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# Effects of Dietary Factors on Production of Adrenal Steroid Hormones

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ALTHOUGH many dietary factors have been considered to be related to the production of adrenocortical hormones, the data are in most instances insufficient to provide conclusive evidence of such relationships. Among the nutrients thought to be concerned with function of the adrenal cortex, those that have been most thoroughly investigated are ascorbic acid, pantothenic acid, and sodium. It is the effect of these nutritional factors on the production of adrenal steroids that will be discussed here.

## ASCORBIC ACID

One of the first observations which related a dietary factor to adrenocortical hormone secretion was the demonstration that vitamin C is present in the adrenal cortex in high concentration.[1] Not only does this gland contain a large amount of ascorbic acid but one of the cardinal manifestations of scurvy in the experimental animal is the occurrence of adrenal hypertrophy.[2] Further investigation demonstrated that as the adrenal cortex responds to corticotropin (ACTH) by secreting its characteristic hormones, there is a concomitant fall in the adrenal ascorbic acid concentration.[3] These facts stimulated interest in the relationship of this water-soluble vitamin to adrenocortical function and suggested that ascorbic acid is implicated in the synthesis of adrenal steroid hormones.

In an effort to determine the role of ascorbic acid in adrenocortical function, an investigation of the secretion of adrenal steroid hormones by scorbutic guinea pigs was undertaken in our laboratory.[4] Guinea pigs were fed a vitamin C-free diet and maintained on this regimen until severe signs of scurvy, such as loss of weight, joint swelling, and bloody diarrhea, were evident. ACTH was then administered to these animals and changes in the number of eosinophils in the peripheral blood were followed. It was found that ACTH administration resulted in a marked decline in circulating eosinophils, thus indicating a release of cortical hormones at a time when the adrenal gland on direct chemical analysis contained only traces of ascorbic acid.

This observation was corroborated by Nadel and Schneider[5] who measured the urinary excretion of hydrocortisone by scorbutic guinea pigs. These investigators found that increased quantities of this steroid hormone were present in the urine of animals that had severe manifestations of vitamin C deficiency. Further confirmation of these findings was ob-

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tained by Done *et al.*[6] who measured the concentration of adrenocortical hormones in the peripheral blood of guinea pigs with severe scurvy. It was demonstrated that a ten-fold increase in blood 17-hydroxycorticoids occurred in vitamin C-deficient guinea pigs when compared to control animals.

As a result of these and similar studies, it is now generally felt that ascorbic acid is not directly concerned with the synthesis and release of adrenocortical hormones but that vitamin C deficiency functions as a nonspecific stress and, as such, results in increased steroid secretion by the adrenal cortex.

## PANTOTHENIC ACID

Attention was first drawn to the relationship between pantothenic acid and the adrenal cortex by the experiments of Morgan[7] and Daft and associates[8] in which it was demonstrated that adrenal hypertrophy, hemorrhage, and necrosis were prominent manifestations of pantothenic acid deficiency. These observations were confirmed and extended by Deane and McKibbin[9] who found, using histochemical technics, that the adrenal cortex of pantothenate-deficient animals has a reduced content of lipid, sudanophilic material, and ketosteroids. The occurrence of these structural alterations stimulated investigation of the functional status of the adrenal cortex in animals deficient in this vitamin.

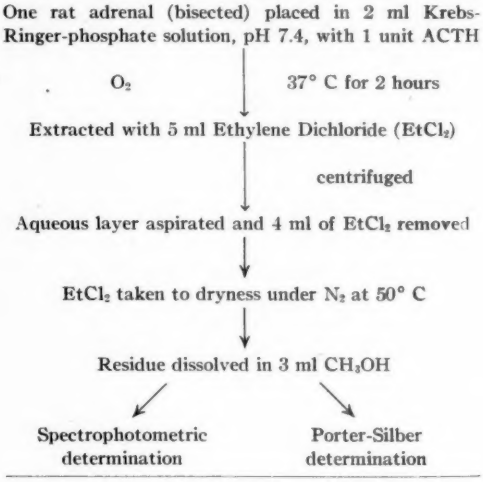
Winters, Schultz, and Krehl[10] studied the effects of administration of ACTH and epinephrine to pantothenic acid-deficient rats and found that these agents failed to produce a significant decrease in the number of circulating eosinophils, whereas cortisone injection was followed by a marked decline in the eosinophil count. These investigators also demonstrated reduced fasting blood sugar levels and increased sensitivity to insulin in deficient animals.[11] Dumm and co-workers[12] made the important observation that cholesterol, a key substance in the synthesis of adrenal steroids, is present in reduced amount in the adrenal cortex of pantothenate-deficient rats following acute stress. Although these investigations, as well as others, suggested that there is diminished secretion of steroid hormones by the adrenals of pantothenate-deficient animals, cortical hormone production was measured by use of indirect methods. For this reason, studies designed to assess the secretory capacity of the adrenal cortex of pantothenic acid-deficient rats have been conducted in our laboratory. In these experiments, steroid secretion of the isolated adrenal glands of pantothenate-deficient and control animals in response to stimulation by ACTH has been studied.[13] The advantage of this approach is that it permits direct chemical determination of hormones secreted by the adrenal cortex.

## METHODS

An outline of the procedure used to measure the secretion of steroid hormones by the isolated rat adrenal gland[14] is presented in Table I. The adrenals are removed from the animal, dissected free of surrounding tissue, weighed, and bisected. Each adrenal is placed

TABLE I

Flow Sheet Describing Recovery and Quantitative Determination of Steroids Secreted by the Isolated Rat Adrenal


One rat adrenal (bisected) placed in 2 ml Krebs-Ringer-phosphate solution, pH 7.4, with 1 unit ACTH
$O_2$
37° C for 2 hours
Extracted with 5 ml Ethylene Dichloride ($EtCl_2$)
centrifuged
Aqueous layer aspirated and 4 ml of $EtCl_2$ removed
$EtCl_2$ taken to dryness under $N_2$ at 50° C
Residue dissolved in 3 ml $CH_3OH$
Spectrophotometric determination
Porter-Silber determination

in a beaker containing 2 ml of Krebs-Ringer-phosphate-glucose solution, pH 7.4. One unit of ACTH is added and the mixture incubated aerobically for two hours at 37° C in Dubnoff metabolic incubator. On completion of incubation, the steroids are extracted from the

aqueous medium with ethylene dichloride and the latter solution taken to dryness. The residue containing the steroids is dissolved in methyl alcohol and aliquots of this solution are utilized for determination of steroid concentration by a spectrophotometric technic and by the Porter-Sibler method. The spectrophotometric procedure detects steroids which possess an alpha-, beta-unsaturated, ketone structure and, thus, measures total production of biologically active steroid hormones by the adrenal gland. The Porter-Silber method detects steroids such as cortisone and hydrocortisone which contain a 17,21 dihydroxy-20-ketone side chain.

Recovery of steroids utilizing this method was determined by adding a known amount of cortisone to the incubation mixture. It was demonstrated that recovery of this steroid was practically complete as measured by both the spectrophotometric and Porter-Silber methods (Table II).

Table III compares the steroid secretion by isolated adrenals of normal rats as measured by the two procedures. It is apparent that the spectrophotometric assay detects more than twice the quantity of steroid material determined by the Porter-Silber reaction. This observation is explained by the fact that the hormone secreted in largest quantity by the rat adrenal is corticosterone, a steroid which is measured only by the spectrophotometric method.

TABLE II

Recovery of Cortisone From Incubation Medium[14]

| | Spectrophotometric method | Porter-Silber method |
|---|---|---|
| 10.3 mcg cortisone added | 10.78 ± 0.19 mcg* (104.7%) 11** | 9.6 ± 0.11 mcg* (93.2%) 12** |

* Mean ± Standard Error of Mean.
** No. of Determinations.

TABLE III

Steroid Secretion by the Rat Adrenal Gland *in vitro*; 19 Determinations in Each Series[14]

| | Spectrophotometric method | Porter-Silber method |
|---|---|---|
| Actual steroid secretion/adrenal | 11.4 ± 0.77 mcg | 4.86 ± 0.24 mcg |
| Steroid secretion per 100 mg adrenal wt | 80.1 ± 5.28 mcg | 34.1 ± 4.58 mcg |

* Mean ± Standard Error of Mean.

## RESULTS

The results of our studies in pantothenic acid-deficient rats demonstrate that cortical hormone secretion per adrenal was significantly reduced when compared to that of pair-fed and ad libitum-fed controls (Table IV). Because there was a marked increase in the ratio

TABLE IV[13]

Steroid Secretion by the Isolated Rat Adrenal Gland

| | Spectrophotometric determination (micrograms of steroid secreted per adrenal) | Porter-Silber determination (micrograms of steroid secreted per adrenal) | Spectrophotometric determination (micrograms of steroid secreted/100 *mg* adrenal tissue) | Porter-Silber determination (micrograms of steroid secreted/100 *mg* adrenal tissue) |
|---|---|---|---|---|
| Group I: Pantothenic acid-deficient animals (25)* | 4.68 ± 0.38 | 2.57 ± 0.18 | 40.1 ± 3.05 | 22.8 ± 1.79 |
| Group II: Pair-fed animals (18)* | 7.67 ± 0 57[a] | 3.57 ± 0.32[a] | 62.7 ± 4.0[a] | 29.2 ± 1.97[b] |
| Group III: Ad lib-fed animals (10)* | 9.66 ± 0.93[a] | 3.50 ± 0.34[b] | 67.3 ± 3.9[a] | 24.6 ± 2.3[c] |

Mean Values ± Standard Error of the Mean:
[a] Difference from Group I statistically significant, P = <0.01.
[b] Difference from Group I statistically significant, P = <0.05.
[c] Difference from Group I not statistically significant.

* Indicates the Number of Animals in Each Group.

of adrenal weight to body weight in the deficient animals, steroid production is also presented as micrograms of steroid secreted per 100 mg of adrenal tissue. When synthesis of adrenal cortical hormones by deficient animals is expressed in this manner, it remains significantly less than that of the pair-fed group. Hormone secretion of deficient animals is also less than that of ad lib-fed controls when measured by the spectrophotometric method, but when determined by the Porter-Silber procedure, there was not a significant difference. It has been mentioned earlier that the Porter-Silber reaction measures less than one half of the total hormone secretion by the adrenal cortex. From these studies we have concluded that, in pantothenic acid-deficient animals the synthesis of adrenal steroids is diminished and that this vitamin is necessary to maintain the functional integrity of the adrenal cortex. These findings are supported by the experiments of Longwell and associates who found that in *in vivo* secretion of adrenocortical hormones by pantothenate deficient rats was diminished.[15]

Since it has been shown that pantothenic acid is essential for elaboration of adrenocortical hormones in response to ACTH, the mechanism by which this vitamin facilitates steroid hormone secretion must be sought. It is known that pantothenic acid does not exist in the free state in the animal body and that the metabolically active form of the vitamin is coenzyme A. Coenzyme A functions as an intracellular carrier of acetyl groups and is necessary for the condensation of these two carbon fragments in the formation of fats, carbohydrates, cholesterol, and steroids. The primary reaction leading to the formation of these complex substances from acetate is the condensation of two molecules of acetyl Co A to form aceto-acetyl CO A. The exact chain of reactions leading to formation of the steroid molecule from this compound is not known, but it is presumed that further condensations take place. Thus, since pantothenic acid is an integral part of coenzyme A, a deficiency leads to depletion of body stores of this coenzyme. It seems reasonable to assume that the end result will be a failure of synthesis of complex substances including the adrenal steroid hormones.

### SODIUM

Restriction of sodium intake has been shown to produce alterations in the adrenal cortex of animals and man. Several investigators[16,17] have demonstrated increased size and decreased lipid content of the zona glomerulosa in sodium-deficient animals and these changes have also been observed in human adrenals.[18] It is also known that sodium deprivation alters hormone secretion of this gland. Luetscher and Axelrad[19] observed that restriction of sodium intake in normal men was followed by increased urinary excretion of aldosterone but that there was no significant change in the excretion of other adrenal hormones. These experiments were of short duration and studies of the effects of prolonged sodium restriction on adrenocortical hormone secretion have not been reported. For this reason, an investigation of the effects of severe, chronic sodium deficiency on adrenal steroid secretion was undertaken in collaboration with Dr. Phyllis M. Hartroft, Department of Pathology, Washington University School of Medicine.[20]

Young, male Wistar rats were fed a sodium-deficient, synthetic diet which was otherwise nutritionally adequate. Pair-fed controls received the same diet to which was added 0.6 per cent sodium chloride. Equal numbers of animals from each group were sacrificed 2, 4, 7, 9, 14, 30 and 60 days after beginning the experimental diets. Serum-sodium, potassium, blood hematocrit, body and adrenal weights were determined when animals were sacrificed. One adrenal from each rat was incubated in a physiologic medium with ACTH according to the method described earlier.[14] The steroids secreted into the medium were recovered and quantitatively determined by the spectrophotometric technic. In addition, paper chromatographic separation of the steroid compounds secreted by the adrenals of deficient and control rats was carried out using the toluene-propylene glycol chromatographic system.[21] The remaining adrenal from each animal was used for histologic study.

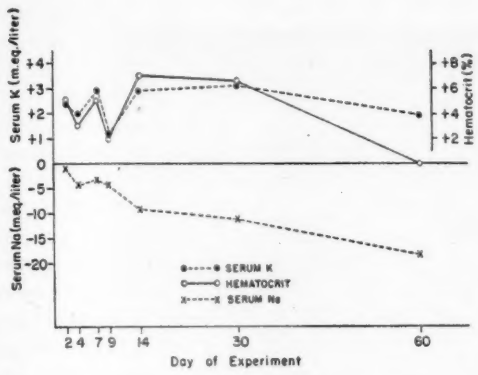



Fig. 1. Serum Sodium and Potassium and Blood Hematocrit of Sodium Deficient Animals Compared with that of Pair-Fed Controls.[20]

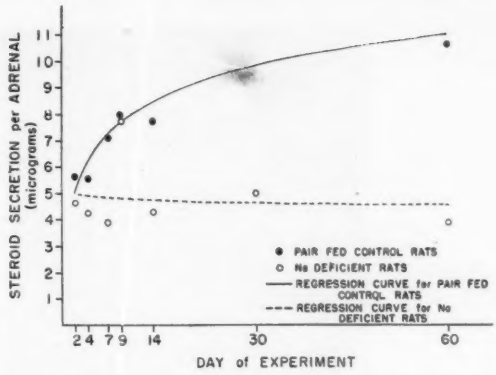



Fig. 2. Total Steroid Hormone Secretion by the Isolated Adrenals of Experimental Animals.

From Eisenstein, A. B. and Hartcroft, P. M.: Alterations in adrencortical hormone secretion induced by sodium deficiency. *Endocrinology* (in press).

The rats consuming the sodium-deficient diet gained weight throughout the period of observation but at a rate which was significantly slower than that of pair-fed controls. Although growth of the animals was decreased by sodium restriction, marked adrenal hypertrophy occurred in these rats (Table V). Serum-sodium levels declined steadily in deficient rats during the 60-day study while the potassium concentration remained elevated. The blood hematocrit was also increased in deficient animals during most of the period of observation (Fig. 1).

Steroid secretion by the isolated adrenals of deficient rats and pair-fed controls is presented in Figure 2. It is apparent that secretion of hormones by the adrenal cortex of control animals increased progressively as the animal grew. Total adrenal hormone secretion of sodium-deficient animals, however, continued at a relatively constant and significantly ($P = < 0.01$) lower rate despite hypertrophy of the gland.

It has previously been shown that isolated adrenals of normal rats secrete four steroids when stimulated by ACTH and that corticosterone is secreted in largest amount.[14] Paper chromatographic separation of hormones secreted by the adrenals of control rats in this study also revealed the presence of four compounds which were present in normal proportion (Fig. 3). Chromatographic analysis of the adrenal secretion of sodium deficient animals on the second, fourth, and seventh days also revealed normal steroid production. By the ninth day, and also on days 14, 30 and 60, increased quantities of aldosterone were secreted by the adrenals of sodium-deficient

TABLE V[20]

Mean Adrenal Weights of Sodium Deficient and Control Rats at Various Intervals After Beginning Experimental Diets

| | Duration of sodium deficiency | Number of animals | Weight right adrenal (*mg*) | Weight right adrenal (*mg*) per 100 *g* body weight |
|---|---|---|---|---|
| Deficient | 2 days | 5 | 8.2 | 11.7 |
| Control | | 5 | 7.8 | 10.4 |
| Deficient | 4 days | 5 | 8.3 | 11.3 |
| Control | | 5 | 8.6 | 10.0 |
| Deficient | 7 days | 5 | 9.2 | 11.5 |
| Control | | 5 | 10.0 | 10.7 |
| Deficient | 9 days | 5 | 9.5 | 10.0 |
| Control | | 5 | 9.2 | 8.6 |
| Deficient | 14 days | 10 | 8.9 | 11.6 |
| Control | | 10 | 8.2 | 7.8 |
| Deficient | 30 days | 10 | 10.3 | 11.8 |
| Control | | 10 | 11.3 | 7.9 |
| Deficient | 60 days | 6 | 15.3 | 9.9 |
| Control | | 6 | 17.0 | 6.4 |

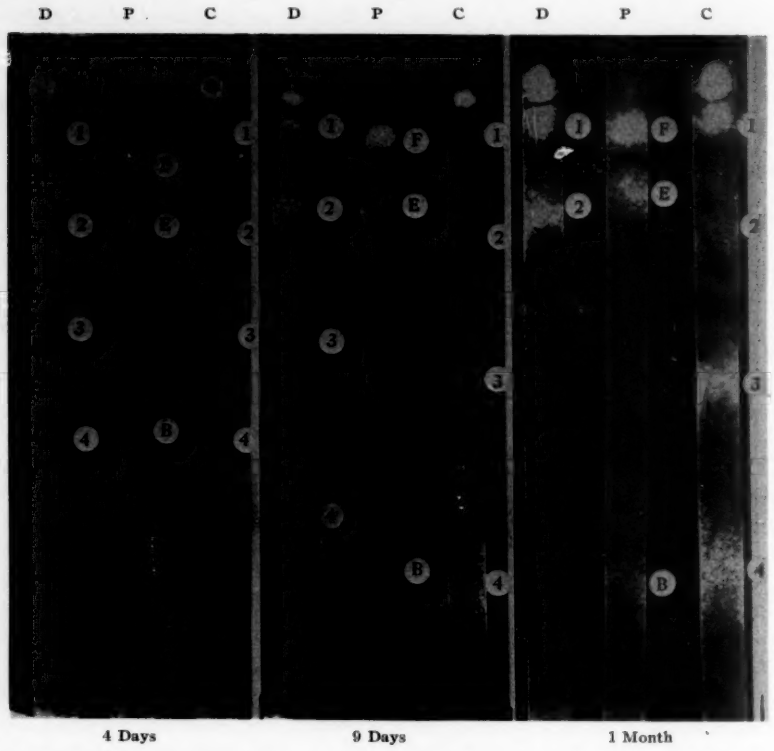



Fig. 3. Chromatograms of the Adrenal Steroids Secreted by Sodium Deficient Rats and Pair Fed Controls.[20]

Each chromatogram consists of three strips labelled D, P, and C. Strip D contains the entire steroid secretion of deficient animals sacrificed on the day indicated and the individual steroids present on the chromatogram are numbered. Strip P is a pilot limb on which hydrocortisone (F), cortisone (E), and corticosterone (B) were placed. Steroid hormones secreted by the adrenals of control animals were placed on Strip C and are also indicated by number.

Previous investigation has shown that steroid 1 is hydrocortisone, steroid 2 is aldosterone, and steroid 4 is corticosterone. The identity of steroid 3 has not been elucidated.[20] (From Eisenstein,A.B. and Hartcroft, P. M.: *Endrocrinology* in press).

animals. The chromatograms also demonstrate that while aldosterone secretion became greater with increasing duration of sodium depletion, the secretion of certain other steroids including corticosterone was greatly diminished (Fig. 3).

Since it was demonstrated in these studies that sodium restriction results in diminished total adrenal steroid hormone synthesis, consideration must be given to the effect of this hormonal change on the ability of the animal to withstand other types of stress. It seems likely that a reduction in adrenocortical hormone secretion as a result of sodium depletion would make the organism less capable of withstanding other stresses such as cold, heat, infection, and injury. Another fact which must be considered is that, in addition to reduction in total hormone synthesis, aldosterone forms a large proportion of the steroids which are secreted by sodium-deficient rats, thus further diminishing the available glucocorticoids. The data obtained in this investigation are not sufficient to substantiate the idea that chronic sodium deficiency may interfere with adaptation of the organism to

stress, but do suggest that further study of this problem should be carried out.

These experiments have also shown that the adrenal cortex in response to sodium depletion is capable of selectively increasing the secretion of aldosterone while decreasing the synthesis of certain other steroid hormones. This has been demonstrated previously by the studies of Singer and Stack-Dunne.[22] It is, therefore, suggested that a *specific* stress may influence synthetic mechanisms concerned with the elaboration of adrenocortical hormones in such a way that the gland secretes increased quantities of an individual hormone. The mechanism by which a specific stress may alter steroid synthesis is not apparent, but it seems likely that factors other than ACTH are involved.

## CONCLUSIONS

The effects of three dietary factors, ascorbic acid, pantothenic acid, and sodium, on production of adrenocortical hormones have been reviewed. Evidence which has been presented indicates that ascorbic acid, although it is present in high concentration in adrenal tissue, is not directly concerned with secretion of adrenocortical steroids. Pantothenic acid, however, has been shown to be intimately related to synthesis of adrenal steroid hormones. It has been suggested that pantothenic acid is essential for elaboration of adrenocortical hormones since this vitamin is an integral part of coenzyme A. It has also been shown that sodium is an important factor in determining hormone secretion by the adrenal cortex since a deficiency of this mineral results not only in increased secretion of aldosterone but leads to diminished production of certain glucocorticoids.

There are many reports which suggest that other nutrients may be concerned with adrenocortical hormone secretion. Although these data do not provide conclusive evidence of such relationships, the studies are stimulating and arouse the interest of investigators in the fields of nutrition and endocrinology. The effect of dietary factors on the production of adrenal steroid hormones remains a fertile area for future investigation.

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# The Effect of Liver Disease on Adrenal Cortical Function

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IT HAS been demonstrated that a number of biochemical modifications of adrenal cortical steroids are made by liver tissue *in vitro*. When cortisol, which appears to be the major secretory product of the human adrenal cortex, is injected intravenously the largest portion of this material, which has been identified, appears in the urine as the hydrogenated derivatives conjugated with glucuronide. There is convincing evidence that these derivatives are formed and conjugated in the liver. One might expect, therefore, that the liver would be an important organ in the regulation of the levels of circulating cortisol in humans.

We have studied the metabolism of cortisol, both endogenous and exogenous, in patients with cirrhosis as well as in subjects with normal liver function. These studies demonstrate that there is altered metabolism of adrenal cortical steroids in patients with liver disease.

## METHODS

The methods which were used in the determinations in these studies involved the Porter-Silber reaction. This reaction depends on the formation of colored phenylhydrazine derivatives of cortisol. The reactive group is the 17, 21-dihydroxy-20-ketone side chain. For convenience, the reacting steroids are usually referred to as 17-hydroxycorticosteroids. The color reaction itself is highly nonspecific. It is therefore essential that for detailed studies great purity of the extracts on which the color reaction is performed be attained. This is accomplished by extraction of plasma or urine with various organic solvents, particularly chloroform, which removes many of the interfering substances. In addition, chromatography on a Florosil column is carried out with nearly all of the extracts. In certain circumstances, it is also useful to partition the material between water and benzene, which further eliminates interfering compounds. The final extracts are essentially free of interfering substances.

Among the steroids, those having the typical side chain of cortisol react with this material in indistinguishable fashion. This means that in addition to cortisol, cortisone and 17-hydroxy-11-desoxycorticosterone (Reichstein's substance S) as well as their hydrogenated derivatives, which still contain the typical side chain, undergo the reaction. For practical purposes, in subjects without adrenal dysfunction, and specifically in the normal subjects and patients with liver disease concerned in this manuscript, the material in the plasma which gives this reaction is cortisol. There is also present a considerable amount of hydrogenated compounds; however, these apparently exist entirely as the glucuronides which are not carried through the extraction procedure.

In the urine, there is a small portion of free 17-hydroxycorticosteroid which, under ordinary circumstances, does not exceed 200 mcg per day. The bulk of the urinary 17-hydroxycorticosteroid is present as glucuronide conjugates and consists of reduced steroids which must be released either by

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glucuronidase or extracted with a different solvent. The details of the methods are described in the papers by Nelson and Samuels[1]; Eik-Nes, Nelson and Samuels[2]; and Brown *et al.*[3]

Two types of special procedures have been carried out in evaluating the adrenal cortical function of these individuals. The first is a test which we have designated the "adrenal cortical capacity."[4] It consists of the continuous intravenous infusion of a maximally stimulating dose of adrenocorticotrophic (ACTH) hormone for a minimum period of six hours. This results in a rapid rise in the plasma level of 17-hydroxycorticosteroids and is useful for evaluating the maximal rate of secretion of which the adrenal is capable.

The second technic consists of the determination of the rate at which cortisol is removed from the plasma when large doses are infused.[3] This process proceeds in a logarithmic fashion; that is, the removal rate is proportional to the level attained. The technic consists of the administration of 1 mg per kg cortisol as an intravenous infusion over a period of one-half hour, following which, values are obtained at intervals for eight hours. By evaluation of these two tests, an estimate can be made of the rate of adrenal secretion as well as the rate of removal of the substance from the plasma.[6]

## RESULTS

### *Plasma Levels of 17-Hydroxycorticosteroids and Response to the ACTH Infusion*

In a series of 17 patients, the morning levels of 17-hydroxycorticosteroids showed a value of 12.6 mcg ± 4.0 per 100 ml of plasma. This compares to a mean of 13.0 ± 3.0 mcg per 100 ml of plasma in a large series of normal individuals. In six patients with liver disease infusion of ACTH produced a progressive rise in plasma 17-hydroxycorticosteroid level. These values averaged 45 mcg per 100 ml at the end of six hours. In 39 normal subjects, a level of 42 mcg was reached in the same period, and the curves were not significantly different. Thus, these examinations showed no evidence of abnormality in the control levels of 17-hydroxycorticosteroids nor in the plasma response produced by the infusion of ACTH.

### *Rate of Removal of Hydrocortisone After a Load Dose*

In contrast to these observations, when hydrocortisone was infused in the standard fashion, the plasma levels at the end of the first hour were 103 mcg per 100 ml as compared to 76 mcg for a control series of 11 normal patients. In both groups, the steroid was removed in a logarithmic fashion, but the half-time in the normal subject was approximately 100 minutes while that in the patients with liver disease was 200 minutes.[3] It is thus apparent that, although the control values of circulating 17-hydroxycorticosteroids are quite normal, these patients do not remove the material from the plasma at nearly as rapid a rate as do normal individuals. Assuming that this also applies at normal plasma levels, it becomes apparent that less steroid would be required to maintain the plasma level at a normal value and that the actual secretion rate was decreased.

### *The Effect of Surgery on Circulating 17-Hydroxycorticosteroid Levels and Its Relation to the Liver*

In a series of 23 patients with major surgery lasting more than two hours, the plasma levels rose spontaneously to a mean of 40 mcg per 100 ml during the six hours following the surgical procedure.[5] It will be noted that this value is essentially identical with that obtained on maximal stimulation of the adrenal cortex over a similar period of time with ACTH. However, when the individual values are observed, the variation is considerably greater. Particularly, values greatly in excess of the maximum level produced in normal individuals by even prolonged ACTH infusion were frequently observed. The administration of ACTH during this period produced an additional marked rise in the plasma levels of 17-hydroxycorticosteroids over the level which had been obtained spontaneously. These values frequently exceeded the maximal values observed after ACTH infusion in normal individuals or in the same patients under control

circumstances. When hydrocortisone was infused in such postoperative surgical patients, higher levels were achieved than those observed in normal individuals or the same patients under control circumstances. There was a tendency for the high levels to persist well beyond the time that was observed in the control studies. When such postoperative patients were studied for their ability to excrete bromsulfthalein, there was a significant correlation between the magnitude of the spontaneous rise in plasma 17-hydroxycorticosteroid level and the increase which occurred in bromsulfthalein retention as a result of the surgical procedure.[6]

## DISCUSSION

In the patients with chronic liver disease, it would appear that the adrenal secretion of cortisol was modified because of a reduced rate of removal of hydrocortisone from the plasma. In these patients, the level of 17-hydroxycorticosteroid was quite normal, but less secretion was required to maintain this level than was required in normal subjects. Similarly, in patients undergoing surgical treatment impairment of hepatic blood flow and hepatic function probably contributed to the rise of 17-hydroxycorticosteroid levels. This was particularly evident in those patients in whom very high levels were observed, which were frequently outside of the range which could be induced by exogenous ACTH. It would appear also that some endogenous stimulation was present in these patients postoperatively.[5]

The observations in the patients with cirrhosis would seem to indicate that the plasma level of cortisol, itself, exerts some controlling influence on the rate of adrenal steroid secretion, probably mediated through the pituitary. However, the apparent stimulation of adrenal secretion by the surgical procedure, even in the presence of elevated levels, indicates that other mechanisms can operate acutely to modify the chronic regulatory system. If we look at the situation in simplified terms, we see that the plasma level or pool is furnished by the secretion by the adrenal cortex. This pool is then removed in a logarithmic fashion by the liver, where the material is degraded to inactive compounds, conjugated with glucuronide in part, and this material is then excreted through the kidney. A very small portion, under ordinary circumstances, of the free circulating steroid also appears in the urine.

Such description of the metabolism of hydrocortisone ignores the fact that this material is a biologically potent one exerting many effects in various tissues. In the light of this fact, there must be an exchange between the plasma pool and the tissue; however, the nature of this tissue phase of the metabolism of cortisol is as yet an obscure one. We have attempted to study the levels in extravascular fluids, where they are nearly always less than in the plasma, unless there is marked inflammation present in the area.[7] With inflammation, these rise to approximately the same as the plasma level. Attempts to analyze for the material in tissue, *per se*, have been fraught with some difficulties, but apparently valid determinations would seem to indicate that even less is present in the intracellular material than is present in the interstitial fluids of the body.

There may also be effects of hepatic dysfunction on the tissue phase of the metabolism of cortisol which is, in reality, the most important phase because it determines the effect on the various metabolic processes of the body. However, at the present time there is no information by which we can critically evaluate this phase of the situation. No gross evidence of excess or deficient adrenal cortical functions as regards hydrocortisone is apparent from the ordinary clinical study of the metabolic processes in the patient with liver disease.

## SUMMARY

Study of patients with cirrhosis of the liver has demonstrated that the plasma levels of 17-hydroxycorticosteroid are normal. However, there is a reduced rate of removal of cortisol from the plasma and therefore less secretion is required to maintain these normal levels.

In the postoperative patients the 17-hydroxycorticosteroid levels rose strikingly. It appears that this change was mediated in part

by hepatic dysfunction and in part by stimulation of the adrenal to secrete 17-hydroxycorticosteroids at sub-maximal rates.

In the evaluation of adrenal cortical function, it is important that the status of hepatic function be considered.

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# The Effects of Endocrine Imbalances on Vitamin Requirements

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STUDIES in this laboratory have indicated that an increased intake of vitamins may be beneficial when either a marked excess or deficiency of certain hormones is present in the body. Thus it was found that an increased intake of vitamin $B_{12}$, thiamine, pyridoxine or the addition of small amounts of vitamin-sparing antibiotics in the diet enhanced the ability of young rats to withstand large doses of cortisone, thyroid substances, thiouracil, diethylstilbestrol, as well as adrenalectomy, thyroparathyroidectomy or alloxan diabetes.[1–4] Growth hormone has also been shown to increase the need for several vitamins[5,6] although it does not appear to increase requirements for vitamin $B_{12}$ (unpublished observations). Under our conditions, neither a vitamin $B_{12}$-deficient, adequate or abundant diet altered the action of growth hormone in increasing growth in hypophysectomized or intact rats. An increased vitamin intake during marked hormonal excesses or deficiencies was usually accompanied by greater appetite and improved efficiency in utilizing food for growth and maintenance purposes. When food consumption was limited, an increased vitamin intake produced little or no beneficial effect.

In vitamin-deficiency states, hormonal imbalances induce more adverse effects on body growth, health, and survival rate than on vitamin-abundant diets. Thus, on a vitamin-deficient diet, the initial inhibition of body growth elicited by administering large doses of such hormones as thyroid substance, diethylstilbestrol or cortisone, is more severe, and even after relatively long periods of time there may be no upward trend in growth. On a vitamin-abundant diet, however, the initial depression of growth is usually followed by a distinct upward trend which may reach the level of rats not given hormones. Such observations indicate that the ability of young rats to adapt themselves to overdosage with hormones depends, in part at least, on an increased intake of vitamins. Similar findings have been made in rats subjected to states of severe hormonal deficiencies.

The biochemical mechanisms by which an increased intake of vitamins benefits young rats given excesses or made deficient in certain hormones remain to be elucidated. Large amounts of hormones may increase metabolic requirements, increase the need to detoxify and eliminate the excess hormones from the body, or stimulate adrenal cortical function. In states of pronounced hormonal deficiencies, on the other hand, an enhanced intake of vitamins may enable essential metabolic re-

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actions to proceed at a rate which is optimal in the absence of hormones, and thus reduces to some extent the need for hormones to increase reaction rates. A sufficient intake of some vitamins may produce desirable pharmacologic effects. Undoubtedly the greater need for vitamins during severe hormonal imbalances is related to the general problem of the effects of "stresses" on vitamin requirements.

It is commonly stated that the rate of body metabolism determines the requirements for B-complex vitamins, and it is widely accepted that a reduction in metabolic rate decreases the need for these vitamins. According to this concept, the need for B vitamins is reduced in hypothyroidism, diabetes mellitus, hypophysectomy or adrenalectomy, since all these states are characterized by a reduction in body metabolism. Yet there is considerable evidence that each of these states is benefited by an increased intake of the vitamin B-complex group. If one considers better health, maintenance of body weight and increased survival rate as desirable in such cases, and also as indices of vitamin "requirements," then it may be concluded that marked hormonal deficiencies increase rather than decrease the need for B-complex and perhaps other vitamins.

It should be emphasized that not all of the harmful effects elicited by marked hormonal imbalances are eliminated following increased vitamin intake. This depends both on the dose of hormone employed and the amounts of vitamins given. Thus the catabolic actions of large doses of thyroid substances or cortisone are not completely overcome even by excessive vitamin consumption. Many of the typical effects of these hormones, such as the increased metabolism evoked by thyroid substances, or the decrease in adrenal weight elicited by cortisone, do not appear to be influenced by increased vitamin intake. In our experiments, we have usually found that 5 to 10 times the normal intake of vitamin $B_{12}$, thiamine or pyridoxine, afforded an optimal protection against the doses of hormones employed. The vitamin intake may need to be even greater in cases of pronounced hormonal deficiences. Dumm and Ralli[7,8] have reported that the amount of pantothenic acid needed to prolong survival in adrenalectomized rats is 40 to 60 times greater than that needed for normal growth. Quantitative vitamin requirements must be determined for each type of hormonal imbalance, although an increased intake appears to be generally helpful in most cases.

The present report is mainly concerned with an attempt to elucidate some of the mechanisms whereby large doses of cortisone increase the need for several of the B group vitamins. This problem is considered to be of particular importance, since an increased secretion of glucocorticoids is a normal consequence of stressful states, and a solution of this problem may provide some explanation of the means whereby "stresses" such as disease or infections increase the need for certain vitamins. The pancreas was included in these studies because there is considerable evidence that large doses of cortisone may stimulate islet function,[9,10] but at the same time interfere with the ability of insulin to increase carbohydrate utilization.[9]

## EFFECTS OF VITAMIN $B_{12}$, ALLOXAN AND CORTISONE ON BODY WEIGHT AND FOOD INTAKE IN YOUNG RATS

Sixty weanling male rats were fed a standard vitamin $B_{12}$-deficient diet[1] for 60 days. They were then divided into six groups of ten each and were treated for 30 days as follows: group 1, no vitamin $B_{12}$; group 2, 200 mcg of vitamin $B_{12}$ per kg of diet; group 3, a single injection of alloxan monohydrate at a dosage of 17.5 mg per 100 g of body weight, given after starving the rats for 72 hours; group 4, the same dosage of alloxan monohydrate and 200 mcg of vitamin $B_{12}$ per kg of diet; group 5, 4 mg of cortisone acetate daily by subcutaneous injection and no vitamin $B_{12}$; group 6, 4 mg of cortisone acetate daily and 200 mcg of vitamin $B_{12}$ per kg of diet. A dose of 200 mcg of vitamin $B_{12}$ was used because it had previously been found that this amount, which is ten times greater than required for normal growth in young rats,[11] produced an optimal counteraction of the catabolic effects of cortisone.

Table I shows the effects of the different treatments on body weight and food intake.

It can be seen that body weight, food intake, and the efficiency of converting food into body weight were greatest in the groups which received vitamin $B_{12}$ (groups 2, 4 and 6). The alloxanized rats on the vitamin $B_{12}$-deficient diet (group 3) showed only half the weight gains and half the efficiency in converting food into body weight as did the vitamin $B_{12}$-deficient rats which did not receive alloxan (group 1). Cortisone elicited an average decrease of about 22.5 g per rat in the vitamin $B_{12}$-deficient animals (group 5), whereas the rats given cortisone and vitamin $B_{12}$ (group 6) gained an average of about 45 g per animal. The decrease in body growth produced by cortisone in both these groups cannot be attributed to reduced food intake, since these animals actually ate more on a body weight basis than the rats in groups 1 and 2 which did not receive cortisone. Thus the large doses of cortisone given interfered with the conversion of food into body-weight gains, although to a much greater extent in the vitamin $B_{12}$-deficient than in the vitamin $B_{12}$-abundant rats.

Previous experiments have shown that vitamin $B_{12}$ supplementation is even more effective when smaller doses of cortisone are administered to young rats. The ability of an increased intake of vitamin $B_{12}$ to counteract several of the catabolic actions of large doses of cortisone, including losses in body weight, have been confirmed in the rat[12,13] and in the mouse.[14]

### EFFECTS OF VITAMIN $B_{12}$, ALLOXAN AND CORTISONE ON BLOOD AND URINARY GLUCOSE AND URINARY NITROGEN

Blood glucose in each of these rats was determined on the 5th, 10th, 20th, and 30th days of the experiment by the micro-method of Folin and Malmros, urinary glucose by the method of Hawkins and Van Slyke and urinary nitrogen by a micro-Kjeldahl procedure.[15] The results are shown in Table II. In the vitamin $B_{12}$-deficient rats (group 1), blood glucose was somewhat lower and urinary nitrogen was somewhat higher than in the vitamin-abundant rats (group 2). Alloxan did not induce as much hyperglycemia and glucosuria in the vitamin $B_{12}$-deficient rats (group 3) as in the vitamin $B_{12}$-abundant rats (group 4), probably reflecting the smaller food consumption of the former. Urinary nitrogen values were about the same in both groups. In the vitamin $B_{12}$-deficient group (5), cortisone increased blood and urinary glucose and urinary nitrogen about 70 per cent more than in the vitamin $B_{12}$-abundant rats (group 6).

The above results help to clarify some of the interactions between cortisone and vitamin $B_{12}$ on body growth and food utilization in these rats. Large doses of cortisone induce hyperglycemia, glucosuria and increase protein catabolism; they also interfere with insulin action and hence with normal glucose utilization.[9] Vitamin $B_{12}$, on the other hand, enhances appetite and increases the transformation of glucose

TABLE I

Effects of Vitamin $B_{12}$, Alloxan, and Cortisone on Body Weight and Food Intake

| Group | Treatment | Body weight, g | | Food intake, g | |
|---|---|---|---|---|---|
| | | Initial | Final | Total | Efficiency (food-gain ratio) |
| 1 | No vitamin $B_{12}$ | 176.7 | 219.8 | 302.6 | 7.02 |
| 2 | Vitamin $B_{12}$ 200 mcg/kg | 169.8 | 257.1 | 402.8 | 4.60 |
| 3 | Alloxan 17.5 mg/100 g | 170.7 | 191.0 | 285.2 | 14.04 |
| 4 | Alloxan + Vitamin $B_{12}$ | 169.6 | 246.1 | 398.2 | 5.20 |
| 5 | Cortisone 4 mg/day, no $B_{12}$ | 170.8 | 148.3 | 257.2 | — |
| 6 | Cortisone + Vitamin $B_{12}$ | 169.9 | 215.3 | 345.2 | 7.60 |

TABLE II

Effects of Alloxan, Cortisone, and Vitamin $B_{12}$ on Blood and Urinary Glucose and Urinary Nitrogen

| Group | Treatment | Initial | 5 days | 10 days | 20 days | 30 days |
|---|---|---|---|---|---|---|
| 1 | No vitamin $B_{12}$ | | | | | |
| | B.G. | 84.4 ± 4.7* | 84.7 ± 4.3* | 84.8 ± 5.6* | 85.1 ± 4.8* | 86.2 ± 5.1* |
| | U.G. | — | — | — | — | — |
| | U.N. | 100.5 | 97.3 | 102.8 | 118.1 | 117.0 |
| 2 | Vitamin $B_{12}$ | | | | | |
| | B.G. | 86.0 ± 5.2 | 95.9 ± 5.1 | 113.9 ± 4.0 | 116.9 ± 3.9 | 118.1 ± 4.6 |
| | U.G. | — | — | — | — | — |
| | U.N. | 108.2 | 90.9 | 72.9 | 77.8 | 75.9 |
| 3 | Alloxan | | | | | |
| | B.G. | 83.8 ± 5.1 | 169.4 ± 10.5 | 183.5 ± 7.9 | 188.9 ± 6.8 | 188.0 ± 6.4 |
| | U.G. | — | 1.64 | 1.92 | 1.86 | 1.54 |
| | U.N. | 107.9 | 128.8 | 131.7 | 126.0 | 130.5 |
| 4 | Alloxan + Vitamin $B_{12}$ | | | | | |
| | B.G. | 82.9 ± 4.4 | 257.5 ± 4.9 | 273.9 ± 8.9 | 277.2 ± 8.1 | 287.5 ± 12.5 |
| | U.G. | — | 2.45 | 2.87 | 2.69 | 2.50 |
| | U.N. | 110.1 | 134.2 | 130.3 | 134.8 | — |
| 5 | Cortisone | | | | | |
| | B.G. | 82.1 ± 7.0 | 185.5 ± 8.9 | 269.4 ± 8.8 | 351.2 ± 15.5 | 380.2 ± 24.3 |
| | U.G. | — | 1.43 | 2.87 | 2.97 | 3.72 |
| | U.N. | 106.0 | 127.7 | 197.4 | 239.1 | 258.8 |
| 6 | Cortisone + Vitamin $B_{12}$ | | | | | |
| | B.G. | 85.4 ± 4.4 | 157.3 ± 3.6 | 202.7 ± 10.1 | 210.3 ± 6.5 | 213.9 ± 14.6 |
| | U.G. | — | 1.24 | 1.82 | 1.94 | 2.06 |
| | U.N. | 100.6 | 115.9 | 152.4 | 157.3 | 162.8 |

B.G. = Blood glucose (mg per 100 ml).
U.G. = Urinary glucose (g/100 g/24 hrs.).
U.N. = Urinary nitrogen (mg/100 g/24 hrs.).
* Standard error of mean.

into body-weight gains, and thereby probably exerts a sparing action on protein catabolism. Long *et al.*[16] and Engel[17] have shown that administration of carbohydrate to cortisone-treated rats counteracted the protein-catabolic effect of the hormone. If large doses of cortisone also increase insulin secretion by the pancreas, it is important to know the relation of vitamin $B_{12}$ to this problem. This is considered in the next experiment.

### EFFECTS OF INSULIN ON BLOOD GLUCOSE IN VITAMIN $B_{12}$, ALLOXAN-DIABETIC AND CORTISONE-TREATED RATS

The same 60 rats used in the previous experiment were used in this experiment. At the end of the 10th, 20th and 30th days of treatment, the rats were starved for 12 hours, initial samples of blood were collected for glucose determinations, and each animal was injected intraperitoneally with 0.2 units of zinc insulin (Iletin). Four hours later blood was collected again for glucose determinations. Previous experiments had indicated that under our conditions, insulin produced a maximum decrease in blood sugar about four hours after injection.

Table III shows that after insulin was injected into the vitamin $B_{12}$-deficient rats (group 1), blood sugar fell an average of 33.0 to 36.8 per cent on each of the three trials, while on the vitamin $B_{12}$-abundant diet (group 2) blood sugar fell an average of about 53 per cent. In the alloxanized rats, insulin was also more effective in reducing blood glucose in the vitamin $B_{12}$-fed rats (group 4) than in the vitamin-deficient animals (group 3), although on a percentage basis the reduction was about the same in both groups. Insulin was least effective in reducing blood glucose in the cortisone-injected, vitamin $B_{12}$-deficient rats (group 5), while an

TABLE III

Effects of Insulin on Blood Glucose in Vitamin $B_{12}$, Alloxanized and Cortisone-Treated Rats

| Group | Treatment | Blood glucose—*mg/100 ml* | | |
|---|---|---|---|---|
| | | 10 days | 20 days | 30 days |
| 1 | No vitamin $B_{12}$ | | | |
| | Before insulin | 84.7 ± 5.6* | 85.1 ± 4.8* | 86.2 ± 2.1* |
| | After insulin | 53.6 ± 3.4 | 57.0 ± 2.4 | 56.5 ± 4.4 |
| | Mg decrease | 31.22 | 28.1 | 39.7 |
| | Per cent decrease | 36.8 | 33.0 | 34.4 |
| 2 | Vitamin $B_{12}$ | | | |
| | Before insulin | 113.9 ± 4.0 | 116.9 ± 3.9 | 118.1 ± 9.5 |
| | After insulin | 53.0 ± 3.1 | 54.7 ± 2.6 | 55.2 ± 3.3 |
| | Mg decrease | 60.9 | 62.2 | 63.9 |
| | Per cent decrease | 53.5 | 53.2 | 53.2 |
| 3 | Alloxan | | | |
| | Before insulin | 183.5 ± 7.9 | 188.9 ± 6.8 | 188.0 ± 1.5 |
| | After insulin | 56.1 ± 4.3 | 50.0 ± 4.2 | 53.4 ± 3.7 |
| | Mg decrease | 127.3 | 138.9 | 134.6 |
| | Per cent decrease | 69.4 | 73.5 | 71.7 |
| 4 | Alloxan + Vitamin $B_{12}$ | | | |
| | Before insulin | 273.9 ± 8.9 | 277.2 ± 8.1 | 287.5 ± 11.0 |
| | After insulin | 101.4 ± 12.3 | 98.7 ± 11.8 | 103.9 ± 11.8 |
| | Mg decrease | 172.5 | 178.5 | 184.5 |
| | Per cent decrease | 62.9 | 64.4 | 64.2 |
| 5 | Cortisone | | | |
| | Before insulin | 269.4 ± 8.8 | 351.2 ± 15.5 | 380.2 ± 16.6 |
| | After insulin | 193.0 ± 9.6 | 275.8 ± 13.3 | 294.0 ± 10.8 |
| | Mg decrease | 76.3 | 75.3 | 86.2 |
| | Per cent decrease | 28.4 | 21.5 | 22.7 |
| 6 | Cortisone + Vitamin $B_{12}$ | | | |
| | Before insulin | 202.7 ± 10.1 | 210.3 ± 6.5 | 213.9 ± 4.3 |
| | After insulin | 54.8 ± 3.8 | 75.2 ± 3.6 | 75.2 ± 3.1 |
| | Mg decrease | 147.9 | 135.1 | 138.7 |
| | Per cent decrease | 72.9 | 64.2 | 64.9 |

* Standard error of mean.

abundance of vitamin $B_{12}$ (group 6) enabled insulin to effectively overcome cortisone resistance.

This experiment is believed to demonstrate that vitamin $B_{12}$ is necessary for maximum insulin action. The need for other B vitamins has also been shown to be increased following the administration of insulin, while a deficiency of B vitamins reduces the action of insulin.[18] It is probable that the ability of insulin to favor conversion of carbohydrate into fat depends in part at least on the presence of adequate vitamin $B_{12}$ intake, since this vitamin has also been shown to be important for this process.[19,21] If large doses of cortisone increase the secretion of insulin by the pancreas, as others have demonstrated, then it follows that the need for B vitamins concerned with carbohydrate metabolism, including vitamin $B_{12}$, should also be increased. This may largely explain the effectiveness of large doses of vitamin $B_{12}$ in counteracting the protein-catabolic actions of excessive doses of cortisone.

### GLUCOSE TOLERANCE IN VITAMIN $B_{12}$, ALLOXANIZED AND CORTISONE-TREATED RATS

Since there is already sufficient evidence that vitamin $B_{12}$ is involved in carbohydrate metabolism, it was of interest to perform glucose tolerance tests in normal, alloxanized and cortisone-treated rats fed vitamin $B_{12}$-deficient or abundant rations. At the end of 33 days of treatment, the same rats used in the previous experiments were starved for 12 hours and

TABLE IV

Glucose Tolerance in Vitamin $B_{12}$, Alloxanized and Cortisone-Treated Rats

| Group | Treatment | Blood glucose, *mg/100 ml* | | | Urinary glucose *g*/100 *g*/6 hr |
|---|---|---|---|---|---|
| | | Initial | 1 hr | 2 hr | |
| 1 | No vitamin $B_{12}$ | 85.22 ± 1.04 | 639.72 ± 23.87 | 417.16 ± 22.56 | 0.52 |
| | Per cent increase | | 650.7 | 389.5 | |
| 2 | Vitamin $B_{12}$ | 124.83 ± 1.50 | 233.33 ± 5.10 | 136.93 ± 2.29 | 0.079 |
| | Per cent increase | | 86.9 | 9.7 | |
| 3 | Alloxan | 187.38 ± 5.35 | 748.82 ± 14.45 | 480.72 ± 20.28 | 0.98 |
| | Per cent increase | | 299.6 | 156.5 | |
| 4 | Alloxan + Vitamin $B_{12}$ | 287.29 ± 13.43 | 755.11 ± 19.74 | 484.25 ± 23.74 | 1.30 |
| | Per cent increase | | 162.8 | 68.6 | |
| 5 | Cortisone | 367.06 ± 13.49 | 884.40 ± 12.42 | 685.82 ± 13.35 | 1.74 |
| | Per cent increase | | 140.9 | 86.8 | |
| 6 | Cortisone + Vitamin $B_{12}$ | 184.60 ± 3.08 | 321.93 ± 10.51 | 230.75 ± 13.45 | 0.68 |
| | Per cent increase | | 74.4 | 25.0 | |

blood samples were collected for initial glucose determinations. A total of 750 mg of glucose in 5 ml of physiologic saline was injected intraperitoneally into each rat, and blood samples were again collected one and two hours later for glucose assays. Urine was also collected for the first six hours after the glucose injection to determine the amount of glucose excreted into the urine. This procedure was repeated after an interval of six days. Since the results were essentially the same in both trials, the results for only one trial will be presented here.

It can be seen in Table IV that the vitamin $B_{12}$-deficient rats (group 1) showed little ability to utilize the injected glucose as compared to the vitamin $B_{12}$-fed animals (group 2). Blood sugar reached levels almost three times as high and total urinary glucose loss was about six times as high in the former as compared to the latter rats. In the alloxan-diabetic rats, a greater increase in blood sugar occurred in the vitamin $B_{12}$-deficient (group 3) than in the vitamin $B_{12}$-abundant rats (group 4), although the amounts of sugar lost in the urine were somewhat higher in the latter group. In the cortisone-treated rats, glucose was elevated to a much higher level in the blood and urine of the vitamin $B_{12}$-deficient (group 5) than in the vitamin $B_{12}$-abundant animals (group 6).

The results shown in the first two groups confirm the report of Ling and Chow[22] that glucose tolerance is much lower in vitamin $B_{12}$-deficient than in vitamin $B_{12}$-adequate rats. These findings also indicate that vitamin $B_{12}$ is important for utilization of glucose in alloxanized rats,

TABLE V

Effects of Different Levels of Cortisone on Body Weight, Food Intake and Excretion of Radioactive Vitamin$B_{12}$ in Urine

| Group | Treatment | Body weight, *g* | | Food intake, *g* | | $Co^{60}$-vitamin $B_{12}$ in urine cps/100 *g*/24 hr |
|---|---|---|---|---|---|---|
| | | Beginning treatment | Final | Total | Food efficiency (Food-gain ratio) | |
| | Vitamin $B_{12}$-deficient | | | | | |
| 1 | Control | 170.0 | 194.0 | 180.8 | 7.53 | 0.5417 |
| 2 | 2 mg cortisone daily | 173.0 | 169.0 | 188.6 | — | 1.1760 |
| 3 | 4 mg cortisone daily | 169.0 | 134.0 | 149.4 | — | 1.8238 |
| | Vitamin $B_{12}$-fed (20 mcg/kg) | | | | | |
| 4 | Control | 193.0 | 246.0 | 238.8 | 4.99 | 0.8265 |
| 5 | 2 mg cortisone daily | 191.0 | 206.0 | 231.0 | — | 0.5949 |
| 6 | 4 mg cortisone daily | 191.6 | 180.8 | 212.1 | — | 1.2391 |

although it cannot be stated with certainty that all islet tissue was destroyed in these rats. The increased interference of cortisone with the utilization of glucose in the vitamin $B_{12}$-deficient rats is demonstrated here by the pronounced hyperglycemia and the large loss of sugar in the urine. On the other hand, when large doses of vitamin $B_{12}$ were fed to the cortisone-treated rats, blood sugar values were only within 25 per cent of the initial level at the end of two hours and urinary sugar losses were greatly reduced.

#### EFFECTS OF DIFFERING LEVELS OF CORTISONE ON URINARY EXCRETION OF RADIOACTIVE VITAMIN $B_{12}$

This experiment was designed to determine further the interactions between large doses of cortisone and vitamin $B_{12}$. Thirty young male rats were initially fed the vitamin $B_{12}$-deficient stock diet (1) for 60 days, and were then divided into six uniform groups of five each. Three groups were continued on the deficient diet while the other three groups were fed 20 mcg of vitamin $B_{12}$ per kg of diet, an amount which has been shown to represent the normal requirement for growing rats.[9] Each of the three groups was further treated for 17 days as follows: group 1, controls, no treatment; group 2, 2 mg of cortisone acetate daily by subcutaneous injection; group 3, 4 mg of cortisone acetate daily. On the 17th day of the experiment, each rat was injected intraperitoneally with 0.1 microcurie of radioactive vitamin $B_{12}$, and a 24-hour urine specimen was collected for counting.

The results are shown in Table V. The effects of cortisone and vitamin $B_{12}$ on body weight and food intake are substantially the same as in previous similar experiments. Insofar as the urinary excretion of radioactive vitamin $B_{12}$ is concerned, it can be seen in the vitamin $B_{12}$-deficient rats (groups 1 to 3) that 2 mg daily of the hormone doubled and 4 mg daily tripled the amount of radioactivity in the urine. On the vitamin-adequate diet (groups 4 to 6), 2 mg of cortisone daily elicited no significant change while 4 mg daily increased the radioactivity in the urine by about 50 per cent. It can also be seen that more of the vitamin was excreted by the vitamin $B_{12}$-adequate controls (group 4) than by the vitamin $B_{12}$-deficient controls (group 1).

These findings are in agreement with the report of Wahlstrom and Johnson[23] that large doses of cortisone increased the urinary losses of microbiologically-measured vitamin $B_{12}$ in baby pigs fed a vitamin $B_{12}$-deficient diet. Chow[20] similarly reported that large doses of cortisone increased the urinary excretion of tracer doses of radioactive vitamin $B_{12}$ in rats. The present experiment indicates that the amount of vitamin $B_{12}$ excreted depends on the dose of cortisone administered. The increased losses of vitamin $B_{12}$ produced by cortisone, particularly in the vitamin-$B_{12}$ deficient rats, probably reflects the interference by cortisone with carbohydrate metabolism and insulin action.

#### INTERACTIONS BETWEEN LARGE DOSES OF CORTISONE AND THIAMINE IN THE RAT

Fifty young male rats were fed a semi-synthetic diet (1) from which thiamine had been omitted for a period of ten days, at the end of which time the rats had reached growth stasis. The rats were then divided into five equal groups of ten each and were treated as follows for 28 days: group 1, no thiamine and no cortisone; group 2, no thiamine and 1 mg of cortisone acetate daily by subcutaneous injection; group 3, 1 mg of thiamine per kg of diet and no cortisone; group 4, 10 mg of thiamine per kg of diet and 1 mg of cortisone acetate daily; and group 5, 1 mg of thiamine per kg of diet and 1 mg of cortisone acetate daily.

The effects of these treatments on body growth and food intake are shown in Figure 1. Growth and food consumption were reduced in all rats by the end of the ten-day depletion period. After this period, the rats which received 1 mg of thiamine (group 3) increased in body weight from 101.7 ± 2.3 g to 151.5 ± 4.1 g each, while the thiamine-deficient rats (group 1) lost an average of about 25 g each in body weight. The latter ate less than half the food consumed by the rats in group 3. An even greater loss in body weight occurred in the thiamine-deficient rats given cortisone (group 2), although food intake was the same as in group 1. These rats were extremely emaciated

and eight of the ten rats showed a pronounced priapism. The addition of 1 mg of thiamine per kg of diet enabled cortisone-treated rats (group 5) to partially overcome the retardation of body growth, while 10 mg of thiamine (group 4) produced an almost complete counteraction of growth inhibition by cortisone. In studies of the interactions between large doses of cortisone and pyridoxine, substantially the same observations were made.[4]

In further experiments, it was found that 20 or 40 mg of thiamine per kg of diet were no losses of nitrogen in the urine and less hyperglycemia than in thiamine-adequate rats. This differs from the effects of cortisone in vitamin-$B_{12}$ deficient rats, in which the urinary nitrogen losses and hyperglycemia were greater than in vitamin $B_{12}$-adequate rats. As in the vitamin $B_{12}$ experiments, however, glucose tolerance and insulin tests showed that thiamine-adequate rats injected with cortisone were better able to utilize glucose than thiamine-deficient rats. The increased consumption of food and utilization of glucose may therefore account for the beneficial effects of extra thiamine in the cortisone-injected rats.

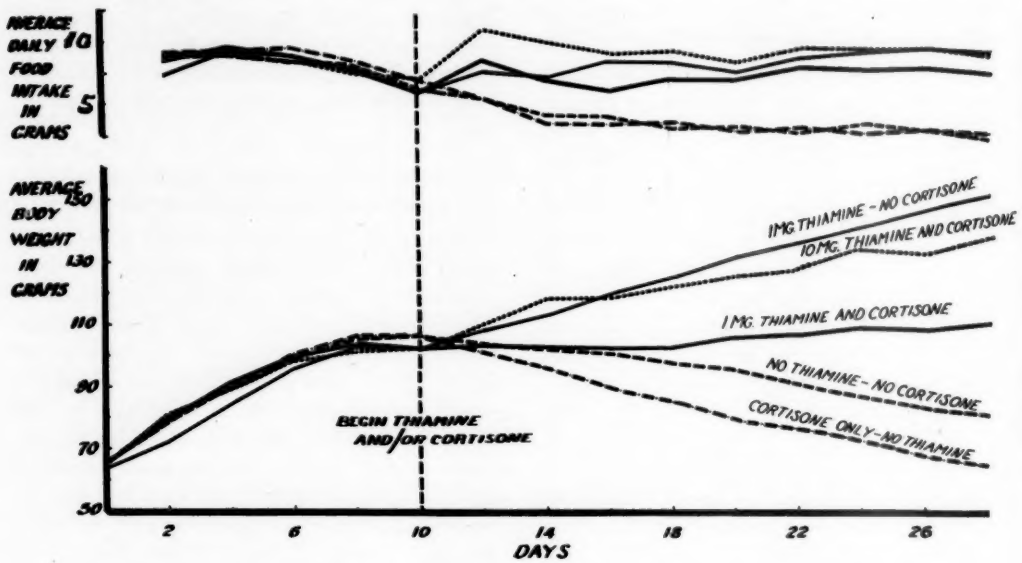


Fig. 1. Effects of cortisone and/or thiamine on body growth and food intake of young rats.

more effective than 10 mg of thiamine. The 10 mg level of thiamine represents ten times the requirement for normal growth in rats,[24] although in a subsequent experiment it was found that 5 mg of thiamine per kg of diet produced an optimal protective action in young rats injected with 1 mg of cortisone daily. The reduction in body weight of the cortisone-injected, thiamine-deficient rats (group 2) can not be attributed to reduced food intake, since these rats ate as much, and on a body-weight basis, more than the rats in group 1.

Biochemical studies revealed that when large doses of cortisone were injected into thiamine-deficient rats, there were smaller

### INTERACTIONS BETWEEN LARGE DOSES OF CORTISONE AND THIAMINE IN GUINEA PIGS

It was considered of interest to determine the interactions between large doses of cortisone and thiamine in the guinea pig, since it has been reported that large doses of cortisone induced a transitory diabetes in this species without inducing losses in body weight.[25] Forty-three male guinea pigs of an average body weight of 354 g each were fed a semi-synthetic diet[26] from which thiamine had been omitted, for a period of 30 days. At the end of this period they

TABLE VI

Effects of Thiamine and Cortisone on Body Weight Gains of Guinea Pigs

| Group | Treatment | Initial body weight, g | Final body weight, g | Weight change, g |
|---|---|---|---|---|
| 1 | No thiamine | 354.0 ± 13.05 | 320.0 ± 50.02 | − 34.0 |
| 2 | 16 mg thiamine | 357.5 ± 21.5* | 501.0 ± 90.3* | +143.5 |
| 3 | No thiamine, cortisone | 352.0 ± 19.5 | 300.0 ± 44.6 | − 52.0 |
| 4 | 16 mg thiamine, cortisone | 355.5 ± 13.05 | 474.0 ± 25.5 | +119.5 |
| 5 | 80 mg thiamine, cortisone | 351.0 ± 14.1 | 481.5 ± 25.7 | +130.5 |

* Standard error of the mean.

were divided into five groups of at least eight per group (eleven in the control group), and were treated as follows for 20 days: group 1, 16 mg thiamine per kg of diet; group 2, no thiamine; group 3, cortisone acetate daily by subcutaneous injection, and no thiamine; group 4, 16 mg thiamine per kg of diet and cortisone acetate; group 5, 80 mg thiamine per kg of diet and cortisone acetate. Groups 3, 4 and 5 received 5 mg of cortisone acetate daily for the first ten days, followed by 10 mg daily for the next ten days.

The effects of the various treatments on body weight gains are shown in Table VI. Unfortunately, accurate measurement of daily food consumption was not possible in these animals and is therefore not presented here. The animals on 16 mg of thiamine per kg of diet (group 1) gained an average of 144.5 g each, while the thiamine-deficient guinea pigs (group 2) lost an average of 34.0 g each. The cortisone-treated animals fed a thiamine-deficient diet (group 3) showed about the same body weight losses as group 2, while the addition of 16.0 or 80.0 mg of thiamine per kg of diet (groups 4 and 5) enabled these animals to gain at about the same rate as group 1.

The effects of the treatments on blood sugar and urinary nitrogen losses are shown in Table VII. There were no significant differences between the thiamine-deficient (group 1) and thiamine-adequate (group 2) animals in blood

TABLE VII

Effects of Thiamine and Cortisone on Blood Glucose and Urinary Nitrogen in Guinea Pigs

| Group | Treatment | Results, mg | | | |
|---|---|---|---|---|---|
| | | Initial | 10th day | 15th day | 20th day |
| 1 | No thiamine | | | | |
| | Blood sugar[1] | 96.0 ± 3.8[4] | 104.0 ± 3.6 | 94.3 ± 4.8 | 93.6 ± 4.6 |
| | Urinary N[2] | 74.0 | 72.4 | 73.5 | 78.0 |
| 2 | 16 mg thiamine | | | | |
| | Blood sugar | 84.0 ± 2.6 | 96.0 ± 6.1 | 108.1 ± 5.8 | 101.4 ± 7.6 |
| | Urinary N | 84.0 | 88.3 | 86.1 | 78.3 |
| 3 | No thiamine, cortisone[3] | | | | |
| | Blood sugar | 92.0 ± 4.2 | 112.0 ± 5.9 | 106.4 ± 6.8 | 84.3 ± 6.3 |
| | Urinary N | 82.0 | 80.7 | 83.5 | 97.3 |
| 4 | 16 mg thiamine, cortisone[3] | | | | |
| | Blood sugar | 104.0 ± 5.1 | 124.0 ± 9.6 | 145.6 ± 10.3 | 193.2 ± 9.2 |
| | Urinary N | 80.0 | 77.2 | 78.3 | 88.1 |
| 5 | 80 mg thiamine, cortisone[3] | | | | |
| | Blood sugar | 96.0 ± 2.8 | 124.0 ± 2.3 | 206.0 ± 9.6 | 208.5 ± 10.2 |
| | Urinary N | 78.0 | 77.5 | 79.2 | 92.3 |

[1] Blood sugar = mg/100 ml.
[2] Urinary N = mg/24 hr/100 g body wt.
[3] For first 10 days, 5 mg cortisone injected daily; for next 15 days, 10 mg cortisone injected daily.
[4] Standard error of mean.

glucose or urinary nitrogen during the experiment. Cortisone did not alter these values when injected into the thiamine-deficient guinea pigs (group 3), but produced gradual and significant increases in blood sugar when injected into thiamine-adequate (group 4) or thiamine-abundant animals (group 5). The increases in blood glucose were greater after 10 mg of cortisone was injected daily than when only 5 mg was given daily. No changes occurred in urinary nitrogen excretion.

The inability of relatively large doses of cortisone to induce weight losses in guinea pigs is in agreement with the report of Hausberger and Ramsey,[25] and differs in this respect from its effects in rats. In the thiamine-deficient guinea pigs, cortisone produced neither hyperglycemia nor increased losses of nitrogen in the urine. Apparently this ineffectiveness of cortisone was the result of decreased appetite induced by thiamine deficiency. Thus, it was found that when the thiamine-deficient, cortisone-injected guinea pigs (group 3) were subsequently placed on a thiamine-adequate diet, blood sugar values rose as in group 4. It is interesting that despite the production of hyperglycemia by cortisone in the thiamine-adequate guinea pigs (groups 4 and 5), there was no increase in urinary nitrogen. This suggests that the hyperglycemia was due to interference with glucose utilization rather than to an increase in gluconeogenesis from protein.

## CONCLUSIONS

We[4,27] have suggested the following hypothesis to explain the interactions between large doses of cortisone and vitamin $B_{12}$: (a) When large doses of cortisone are injected into young rats deficient in this vitamin, there is a pronounced increase in gluconeogenesis from protein, hyperglycemia and an increase in insulin secretion. Cortisone and the vitamin $B_{12}$ deficiency interfere with normal insulin action, increasing the hyperglycemia and glucosuria, and leaving little carbohydrate available for anabolic purposes. These lead to inhibition of body and hair growth and losses in weight of lymphoid tissues. (b) When large doses of cortisone are given to young rats fed an abundant intake of vitamin $B_{12}$, there is an increase in food consumption and availability of carbohydrate. These stimulate insulin secretion and thereby promote carbohydrate utilization for energy and storage purposes, with a resultant reduction in protein catabolism by cortisone, leaving more protein as well as carbohydrate available for body building purposes. Presumably some, but not all, of the interactions between large doses of cortisone and thiamine or pyridoxine would be similar in nature.

The above hypothesis cannot be accepted without reservation and may be inaccurate in some of its assumptions. Thus it is difficult to understand why, if large doses of cortisone produce an increase in insulin secretion, there is no increase in body retention of B-vitamins essential for carbohydrate metabolism. It has been shown that large doses of cortisone produce an increase in urinary excretion of vitamin $B_{12}$, particularly in vitamin $B_{12}$-deficient rats, while the metabolism of riboflavin, pyridoxine, pantothenic acid, biotin, niacin and folic acid have been reported to be unchanged by cortisone administration.[28,29] The metabolism of several of these vitamins was also not altered by adrenalectomy.

The possibility exists that large doses of cortisone may not increase insulin output in animals whose diet is deficient or barely meets minimum vitamin requirements, in addition to inhibiting insulin action. Greater appetite has invariably accompanied increased vitamin administration to cortisone-treated rats, and a sufficient intake of food may be essential to elicit a maximal increase in insulin secretion and action. When large amounts of vitamin $B_{12}$ or thiamine were given to cortisone-treated rats whose food intake was limited to that of vitamin-deficient rats, there was no counteraction of the catabolic actions of cortisone. Although the benefits of an increased intake of vitamin $B_{12}$ or thiamine in cortisone-treated rats are associated with increased food consumption and utilization, it has been shown that vitamin $B_{12}$ renders cortisone less effective, and thiamine renders it more effective in increasing nitrogen and urinary losses. This suggests that there are important differences in the interactions between cortisone and each of these two vitamins. It is obvious that fur-

ther work will be necessary to clarify these and other interrelationships between hormones and nutrients.

### ADDENDUM

Contrary to the reports of negative effects of cortisone on the metabolism of certain B vitamins,[28,29] other workers have recently noted that prednisone and ACTH may alter the urinary excretion of several of these vitamins. When rats on an adequate diet were given large doses of prednisone, urinary elimination of vitamin $B_{12}$ was increased, while thiamine and pantothenic acid excretions were decreased, with folic and folinic acid remaining unchanged (Ginoulhiac, E., and Nani, S.: Ricerche sperimentali sulla eliminazione urinaria di tiamina, di cobalamina, di acido pantotenico, di acido folico e di acido folinico in ratti trattati con prednisone. *Acta Vitaminologica* 4:157, 1956). The administration of ACTH to human patients resulted in a decrease in urinary excretion of thiamine, and it was suggested that sufficient thiamine be given to paients during ACTH or cortisone treatment (Aceto, G., Li Moli, S., and Panebianco, N.: Influenza dell' ACTH sull'eliminazione urinaria di vitamina $B_1$. *Acta Vitaminologica* 4:175, 1956).

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# Newer Concepts of the Action of Insulin

*By* WILLIAM C. STADIE, M.D.*

SINCE the discovery of insulin, thousands of research workers have concerned themselves with experiments designed to show the metabolic defect in diabetic organisms and the mode of action of insulin upon mammalian metabolism. The problem is still obscure but a pattern begins to reveal itself and in this brief discussion I shall undertake to describe this pattern as I see it. Many hypotheses have been formulated to explain why the early steps in the metabolism of glucose are impaired in diabetes. The possible role of the cell membrane was neglected until Hoeber,[1] in 1927, proposed a "membrane theory of diabetes." He called attention to the possibility that certain pathologic relationships in cellular metabolism might be due to disturbances of normal permeability of cells. He said: "One thinks that an abnormal constitution of the cell membrane (perhaps of the liver cell) does not allow the simple sugars to reach their normal focus of breakdown. If one considers the above possibility it is possible to substitute for the present theory of diabetes, which is based upon concepts of abnormal enzymatic activities, another viewpoint: the membrane theory of diabetes." Hoeber himself presented no experimental data to support this hypothesis and some 25 years passed before two investigators, Levine[2] of Chicago and Ross[3–5] of London studied the possible role of permeability factors in diabetes, each approaching the subject from different points of view. Levine selected the eviscerated dog as the experimental preparation for study. He selected sugars, for example galactose, which were essentially inert in the eviscerate preparation. The resultant level of blood galactose following intravenous injection enabled him to calculate the volume of distribution of that sugar. The results of his experiments are illustrated schematically in Figure 1.

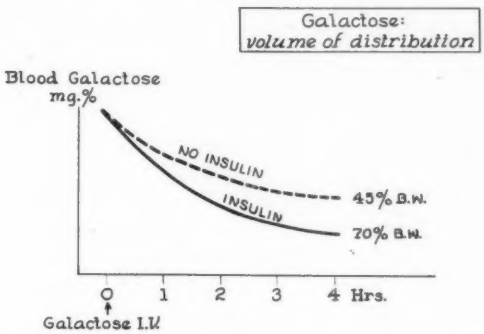


Fig. 1. Effect of insulin on glucose transport (eviscerate-nephrectomized dog). Data of Levine *et al.*, 1950.

In the absence of injected insulin, blood galactose levels became constant and the volume of distribution of galactose was calculated to be of the order of 45 per cent of the body weight. In the same preparation when insulin was simultaneously injected with galactose the final blood levels indicated a volume of distribution of 70 per cent of the body weight, essentially that of body water. In similar experiments Levine studied other sugars and he divided them into two groups: (1) those which were nonresponsive to insulin in that the volume of distribution in the eviscerate preparation was unchanged by the simultaneous injection of insulin; (2) those sugars which were insulin-responsive in that the volume of distribution was increased. In this latter group were placed *d*-galactose, *l*-arabinose and *d*-xylose. Levine called attention to the fact that the structures of these sugars with respect to carbons 1, 2, and 3

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were similar and hence he concluded that the entrance of sugar into muscle "is governed by a transfer system which is specifically adapted with respect to chemical structure." Glucose was included in the insulin responsive group because its structure was similar to that of those sugars which were determined to be insulin-responsive.

Ross[3–5] at the Institute of Ophthalmology (London) approached the subject in a different way. He studied the rate of penetration into the aqueous humor of a number of substances shown in Figure 2. The rate of penetration was calculated in the form of a permeability constant shown on the ordinate and as the abscissa he used the relative lipid solubility, that is the solubility of the substance in alcohol as compared to its solubility in water. He

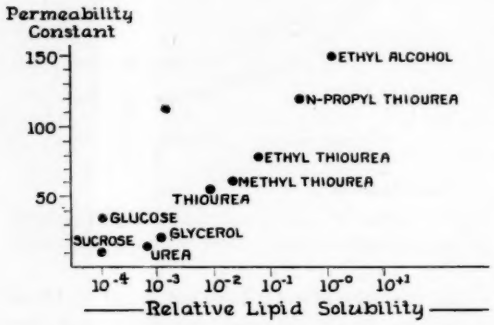



Fig. 2. Relation between transport across blood-aqueous barrier of the eye and the blood lipid solubility of solutes. Data of Ross, 1951.

found a striking correlation between these two variables and Ross concluded that these relations are in accord with the current concepts of cellular membrane structure which as originally postulated by Overton have lipid characteristics. The greater the lipid solubility of a metabolite the more readily does it enter into the cell. However, Ross observed that glucose, as is evident in the figure, was an exception having a relatively low lipid solubility but a relatively high permeability constant. Obviously some other factor must be involved in the ready penetration of glucose into the aqueous humor through the ciliary body and it was natural to suppose that insulin plays some role here. Testing this hypothesis experimentally, Ross found data which are illustrated in Table I.

TABLE I

Transfer of Glucose Across Blood-Aqueous Barrier in Rabbit Eye. Data of Ross, 1952

| | 1000 $k_{in}$ |
|---|---|
| Normal | 199 ± 36 |
| Alloxanized (Blood glucose 400–500 mg. 100 ml) | 102 ± 10 |
| Decrease | 97 ± 38<br>+ = 2.5 |

In the normal rabbit the permeability constant across the blood aqueous barrier was of the order of 200 in Ross' units but in the alloxanized preparation, characterized by high blood sugar levels, the permeability constant was approximately 50 per cent of that of the normal. Ross further observed that treatment of the alloxanized diabetic rabbit with insulin restored the permeability constant to normal values. On the basis of these findings Ross concluded that at least one function of insulin was to influence change in the permeability of the ciliary body to glucose.

Park[6,7] and his co-workers were the first to obtain direct evidence that the transport of glucose across cell membranes was accelerated by insulin. They used the rat diaphragm, the heart, or the skeletal muscle *in vitro* and by appropriate methods were able to determine the intracellular free glucose, that is glucose uncombined with phosphate as a hexose phosphate.

Figure 3 shows typical experiments obtained by Park. Using paired rat diaphragms at 37° C and isotopic glucose in the medium he showed that in the presence of insulin, the concentration of free glucose, i.e., sugar uncombined with phosphate, within the diaphragm proper was higher than in the absence of insulin. From many experiments of this type, Park and his co-workers concluded: "An action of insulin on glucose uptake is to accelerate a step concerned with the transport of glucose into the tissues. This step antecedes and is distinct from glucose phosphorylation by the hexokinase reaction."

This so-called transport hypothesis of the action of insulin with respect to glucose in the periphery has been amply supported by work of other authors. However, it cannot be generalized to apply to all other types of tissues. In fact, as I shall point out subsequently, there is good evidence to indicate the contrary.

### DEFECTS OF GLUCOSE METABOLISM IN THE DIABETIC LIVER

There is still considerable divergence of opinion regarding the defects of glucose metabolism in the diabetic liver. Table II presents a summary of metabolic defects of glucose metabolism in the diabetic liver determined from experiments in the alloxanized rat. Much of this evidence has been obtained by the study *in vitro* of liver slices from the diabetic rats or cats using isotopic glucose. In summary: Table II shows that (1) there is a diminished uptake of glucose; (2) a diminished oxidation of glucose by the liver; (3) a diminished conversion of glucose to glycogen, hence a diminished glycogen storage; (4) a depression of the ability of the liver to phosphorylate glucose; and (5) a markedly increased new formation of glucose, particularly from unknown precursors, presumably protein. One can conclude that the evidence shows that a metabolic block in the early stages of hepatic metabolism of glucose exists in the diabetic. But there is much evidence to indicate that a second and independent metabolic block exists in the diabetic liver affecting adversely the complete oxidation or synthesis of fatty acids. In other words, we have two distinct metabolic derangements in the diabetic liver: (1) pertaining to glucose metabolism; and (2) pertaining to fatty acid synthesis.

TABLE II

Fatty Acid Metabolism of Slices of Liver from Diabetic Rats. Data of Chaikoff *et al.*, 1951

| | |
|---|---|
| Glucose uptake | Decreased |
| Conversion to $CO_2$ | Decreased |
| Glycogen formation | Markedly decreased |
| Glucose output | Markedly increased |
| Glucose from unknown sources | Markedly increased |

A striking phenomenon must be emphasized here. No one to my knowledge has ever demonstrated *in vitro* a reversal toward normal of these metabolic defects by the addition of insulin to the equilibrating medium when liver slices are studied *in vitro*. In contrast, in the normal liver slice the effects of insulin upon fatty acid synthesis from lactate, acetate, or from pyruvate are easily demonstrable. In contrast, the response to insulin in muscle from normal and diabetic rats is immediate. This appears to me to be at the base of some fundamental difference in the mechanism of insulin reaction in the two types of tissue.

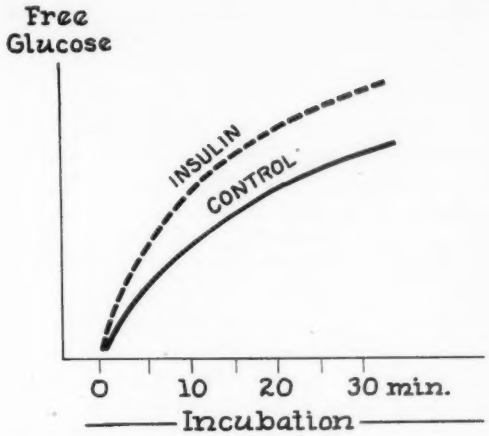
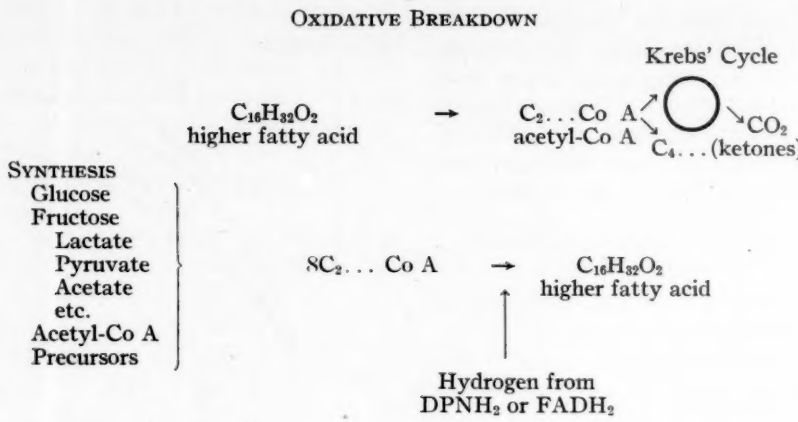



Fig. 3. Effect of insulin on intracellular free glucose in rat diaphragm. Temperature equals 37° C; glucose equals 2,000 mg/100 ml. Data of Park *et al.*, 1956.

### FATTY ACID METABOLISM IN THE DIABETIC LIVER

Our present knowledge of fatty acid synthesis or fatty acid breakdown is presented in a highly schematic way in the following formula devised according to the author's data (p. 396).

Higher fatty acids, for example, palmitic acid, a 16-carbon fatty acid, is broken down by successive beta oxidation. This process involves three steps: condensation with coenzyme A, oxidation at the $\beta$ carbon, and a splitting off of two carbons to form acetyl-coenzyme A. The acid is now shorter by two carbons. A repetition of this process results in the complete breakdown of the higher fatty acid in acetyl-CoA. In the liver, two fates of the acetyl-CoA are of immediate in-




terest: (1) oxidation through the Krebs' cycle to carbon dioxide, or (2) combination of two acetyl-CoA molecules to form a 4-carbon ketone which is not further metabolized in the liver. The synthesis of higher fatty acids, e.g., palmitic acid, is indicated in the second half of the schema. There are certain precursors of acetyl-CoA. These are glucose, lactate, pyruvate, fructose, or acetate. Acetyl-CoA molecules, by a series of condensations and reductions, are built up step by step into longer and longer chains until the final product, palmitic acid is synthesized. But it is important to emphasize that the path of synthesis is not entirely the same as the path of oxidation.

Stetten and Boxer[8] were the first to show that in the intact diabetic animal lipogenesis is seriously impaired. The impaired lipogenesis observed in the intact diabetic animal can be demonstrated with great convenience using liver slides *in vitro*.

Table III shows data taken chiefly from Chaikoff's work[9] which were obtained by equilibrating liver slices from diabetic rats with media containing various metabolites. In all cases, the metabolites were uniformly labeled with isotopic carbon, enabling an identification of the carbon in carbon dioxide or in higher fatty acids. With glucose as the metabolite, no radioactivity is found in the $CO_2$, a result fully to be expected and indicating the presence of the first metabolic block at an early stage of glucose metabolism. In addition, there is no fatty-acid synthesis from the glucose indicated by the zero in the last column. With fructose as the metabolite, the production of $CO_2$ is the same as that observed in the normal rat. Now this means that the formation of acetyl-CoA from fructose is unimpaired in the diabetic. However, there is no synthesis of higher fatty acids from fructose. The same findings are observed with acetate. When isotopic lactate was used, Chaikoff found

TABLE III

Departures from Normal of the Glucose Metabolism of Liver Slices from Alloxanized Rats. Combined Data from the Literature

| Isotopic Nutrilities | Recovered as $CO_2$ | Isotopic carbon as higher fatty acid |
|---|---|---|
| Glucose | 0 | 0 |
| Fructose | Normal | 0 |
| Lactate | Normal | 0 |
| Pyruvate | Normal | 0 |
| Acetate | Normal | 0 |

normal amounts of $CO_2$ production again indicating a normal production of acetyl-CoA from this metabolite. But no fatty-acid incorporation was observed. Isotopic pyruvate gave essentially the same results. Except in the case of glucose, these data clearly mean that we have all the conditions necessary for fatty-acid synthesis, namely, acetyl-CoA formation and a source of hydrogen for purposes of reduction. Nevertheless, higher fatty acids are not synthesized. The conclusion seems inescapable that, independent of glucose me-

tabolism, there is a second block in the diabetic liver impairing the synthesis of fatty acids. From the summation of this evidence, Chaikoff[10] concluded: "A second biochemical block must therefore be postulated for the diabetic liver, and the observations with $C^{14}$-acetate indicate that it lies in the path of conversion of a '2-carbon-like' compound to fatty acids." This metabolic block, so clearly demonstrated in the liver slice of the alloxanized rat (Table III) may be eliminated in two ways: (1) by the prior dietary administration of fructose and (2) by the prior administration of insulin. But it is important to note that the results are not the same: Prior fructose feeding restores the ability of the liver slice to synthesize fatty acids from acetate, lactate, and pyruvate but does not restore the loss of the ability of the liver slice to oxidize glucose or incorporate it into fatty acid. Insulin treatment, on the other hand, eliminates *both* metabolic blocks.

These data from Chaikoff's laboratory must be considered in relation to data obtained in studies on the metabolism of liver slices from depancreatized cats obtained in our laboratory and the laboratory of Gurin. Consider the data obtained by Stadie, Zapp, and Lukens[11] on the formation of ketone bodies by cat-liver slices (Table IV). Note the high rate of ketone formation (1,200) in the case of the depancreatized cat as compared to normal (240). In the Houssay preparation ketone formation is reduced to practically zero. On the other hand, the rate of incorporation by liver slices of acetate into long chain fatty acid[12] in the depancreatized cat is essentially zero but in the Houssay cat it is restored to normal.[5] This can only lead to one conclusion, namely, that the oxidative breakdown of higher fatty acids to butyryl or acetyl-CoA from which the ketones are formed is unimpaired in the diabetic state; and second that synthesis of fatty acids from acetyl-CoA is impaired. A corollary of these two conclusions is that the two pathways, namely, oxidative breakdown, and the synthesis of fatty acids, are not the same.

TABLE IV

Fatty Acid Synthesis from Acetate and Ketone Formation by Liver Slices from Normal, Depancreatized and Houssay Cats

| | Acetate incorporated by cat liver slices into long-chain fatty acids Brady, Lukens, and Gurin (1951) | Ketone formation by cat liver slices, Stadie, Zapp, and Lukens (1940) |
|---|---|---|
| | Mg/100 mg, Mean | Micromoles/kg cat, Mean |
| Normal Cats | 2–8 | 240 |
| Depancreatized Cats | 0–0.7 | 1,200 |
| Houssay Cats | 5 | 85 |

Further experimental evidence that there are two pathways of fatty acid metabolism, one of which is impaired in the diabetic, is indicated by the data shown in Table V from experiments by Shaw from Gurin's laboratory.

TABLE V

Fatty Acid Synthesis by Hepatic Mitochondria from Normal or Diabetic Rats. Data of Shaw and Gurin, 1956. Added Cofactors: Mg; Citrate; DPN. Precursor of Acetyl CoA: Isotopic Pyruvate.

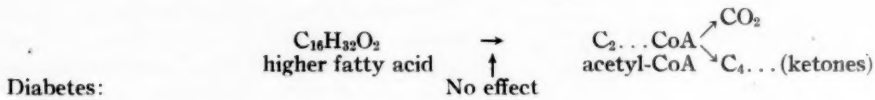
| Source of mitochondria | Further additions | Fatty acid synthesis |
|---|---|---|
| Normal rat liver | Normal liver supernatant | Yes |
| | None | No |
| | Butyryl-CoA | Yes |
| Diabetic rat liver | Normal liver supernatant | Yes |
| | Diabetic liver supernatant | No |
| | Butyryl-CoA | Yes |

Shaw and Gurin[13] studied fatty acid synthesis by mitochondrial preparations made from livers of normal or depancreatized rats. The system used consisted of four parts: (1) washed mitochondria, either intact or lysed; (2) the supernatant from homogenized liver spun at 100,000 *g* to remove all particulate matter; (3) certain cofactors such as Mg, citrate, coenzyme I (indicated as DPN) and (4) a precursor of acetyl-CoA, namely, isotopic pyruvate. The figure shows that in the case of mitochondria from normal rat liver, fatty acid synthesis from the isotopic pyruvate occurs when normal liver supernatant is included in the system. But synthetic butyryl-CoA alone gives the same result. Synthesis of higher fatty acids from the pyruvate then occurred at a rate comparable to that observed

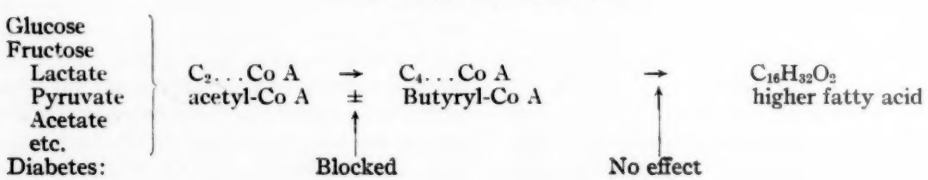
with normal liver supernatant. With mitochondrial preparations made from diabetic rat liver, the addition of supernatant prepared from the diabetic liver resulted in no fatty-acid synthesis from pyruvate. With normal liver supernatant added, fatty-acid synthesis did occur. The same, however, was observed with a butyryl-CoA alone. In other words, diabetic mitochondria behave like normal mitochondria when butyryl-CoA is present. On the basis of his experiments, Shaw concluded that "butyryl-CoA appears to be able to replace the supernatant fraction of both the normal and diabetic water-soluble systems. It seems reasonable, therefore, to suggest that the diabetic liver is unable to convert pyruvate, acetate or acetyl-CoA to fatty acids, because it cannot convert acetyl-CoA to butyryl-CoA. This involves two oxido-reduction steps which in fatty acid are: (1) the reduction of acetoacetyl-CoA to beta-hydroxy-butyryl-CoA and (2) the hydrogenation of crotonyl-CoA to butyryl-CoA. The former step requires reduced DPN and the latter, reduced FAD. This failure in the diabetic liver which appears in the supernatant fraction of the liver homogenate may be due to the inability of the soluble enzyme system to supply one or both of these two cofactors."

Following is the author's schema (1956) of fatty acid metabolism in the diabetic animal based upon the evidence that we have been discussing.

PATHWAY I—OXIDATION




PATHWAY II—SYNTHESIS




Pathway I indicates the steps involved in oxidation. A higher fatty acid containing, let us say, 16-carbon atoms is broken down by reactions involving successive condensation with coenzyme A and beta codation. A stepwise formation of acetyl-CoA occurs. In the liver the acetyl-CoA is either oxidized in the Krebs' cycle to $CO_2$ or by condensation of two molecules of acetyl-CoA produces a 4-carbon ketone body. As we have seen from our data with the diabetic cat the production of ketone bodies proceeds at a very rapid rate and therefore the enzyme system and the necessary cofactors for this pathway are unimpaired by insulin lack.

Pathway II shows the synthesis of higher fatty acids from acetyl-CoA precursors such as glucose, fructose, lactate, pyruvate, acetate, etc. As we have seen, acetyl-CoA formation (except from glucose) is unimpaired in diabetic liver slices, the ability of acetyl-CoA to engage in the successive reaction of condensation and reduction with the final production of higher fatty acids is impaired. The work of Shaw makes it reasonable to assume that the jump from acetyl-CoA to butyryl-CoA is the locus of the diabetic defect.

Returning for a moment to Table IV, the evidence here indicates strongly that the system which functions to bridge this gap between acetyl-CoA and butyryl-CoA is impaired in the diabetic state because of the inhibitory action of pituitary factors which become manifest owing to the lack of insulin. In the hypophysectomized depancreatized animal, the incorporation of acetate into higher fatty acids is restored to the normal state.

In other words, in the Houssay cat the removal of the pituitary factor has restored the ability of the diabetic liver slice essentially to its full synthesizing capacity with respect to fatty acids. Note further that in the Houssay preparation ketone formation falls to zero indicating that the normal balance between synthesis and oxidation of fatty acid has been restored and homeostasis of fat metabolism of the liver has been re-established. It is a possible conclusion then to state that the enzyme system bridging the gap from $C_2$ to $C_4$ in fatty acid synthesis must be under balanced control of both insulin and pituitary factors.

### PHOSPHATE METABOLISM IN THE DIABETIC ANIMAL

Phosphate metabolism in the diabetic state has received much attention in the literature. It is now well established that the oxidation of certain metabolites through the Krebs' cycle brings about the incorporation of inorganic phosphate into energy-rich types of organic combination, for example adenosine triphosphate. These become sources of chemical energy for a wide variety of metabolic activities. Much evidence in the literature makes it reasonable to assume that a deficient formation of these energy-rich phosphate bonds may play a role in the metabolic defect of the diabetic animal.

It is fairly well established that oxidative phosphorylation occurs exclusively in the mitochondria. Vester[14] in our laboratory studied the possible involvement of the defect in oxidative phosphorylation in the mitochondria by using mitochondria prepared from the livers of depancreatized cats. I show a summation of his data in two figures. Figure 4 compares oxidative phosphorylation by isolated hepatic mitochondria obtained from normal and diabetic cats. The first section of the figure shows the ATP formation by the mitochondria using pyruvate as a source of energy. Note that the rate of formation of ATP in the diabetic mitochondria is significantly diminished. The middle section shows the difference in oxygen uptake but more significant is the section on the right hand side indicated by the P:O ratio. The P:O ratio is the ratio between the amount of ATP formed as compared to the oxygen uptake. This ratio (on right of the figure)

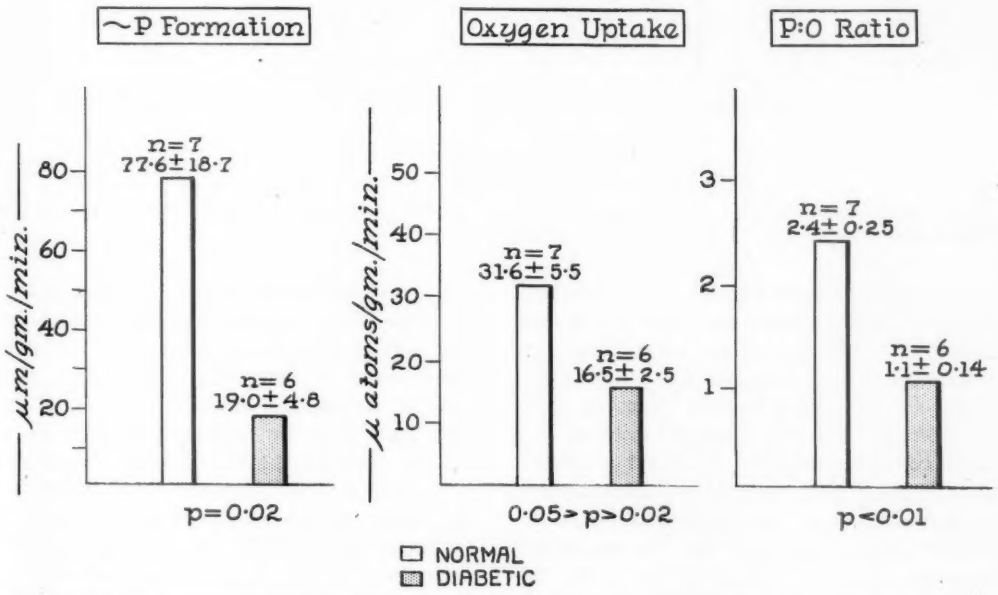



Fig. 4. Oxidative phosphorylation by hepatic mitochondria from normal and diabetic cats. Data of Vester and Stadie, 1956.

measures the efficacy of oxidative phosphorylation by the mitochondria. The P:O ratio of the diabetic mitochondria is significantly diminished as compared to the normal value.

As seen in Figure 5 Vester showed that the impairment of these manifestations of oxidative phosphorylation can be reversed by prior treatment of the diabetic cat with insulin. His experiments were done in the following way. Two or three days after pancreatectomy a liver biopsy was done. Mitochondria were prepared and tested as before. Then the cat was vigorously treated with insulin for a period of two or three days, restored essentially to normal and a second specimen of liver obtained. As before, mitochondria were prepared and assayed for oxidative phosphorylation. The conclusions drawn from the data are obvious. In the left hand column, the solid column indicates the total oxidative phosphorylation as measured by ATP formation in the diabetic state; the hatched column in the restored state. It is clear that the insulin has restored oxidative phosphorylation in the isolated mitochondria essentially to normal. The same conclusion can be drawn with respect to the P:O ratio which is shown in the right hand side of the slide.

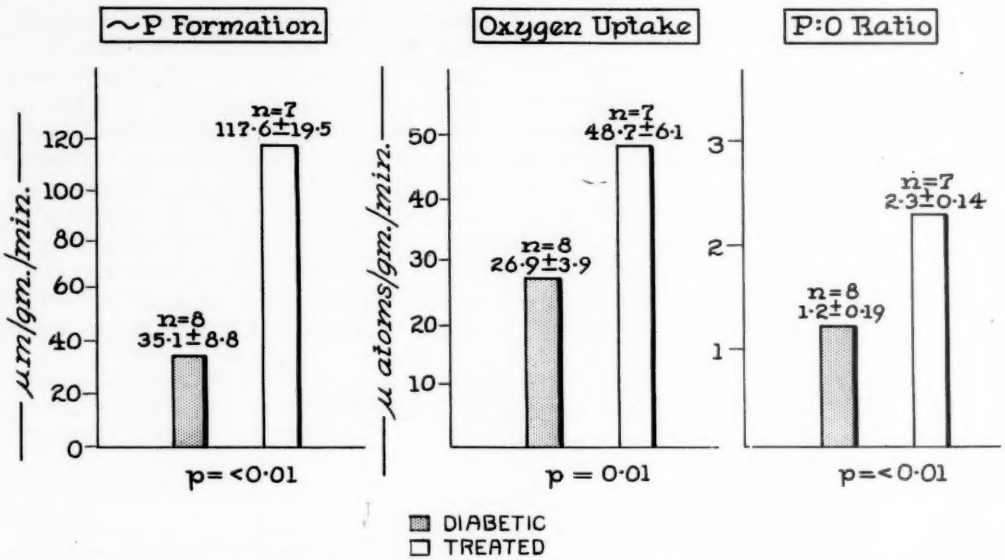



Fig. 5. Restoration to normal of the oxidative phosphorylation by mitochondria prepared from livers of insulin treated depancreatized cats. Data of Vester and Stadie, 1956.

The summation of Vester's evidence which I have cited here, together with that in the literature, indicates that oxidative phosphorylation is impaired in the diabetic status and this impairment cannot be ignored as a possible factor for departures of both fat and carbohydrate metabolism from normal.

### BINDING OF INSULIN[14-19]

The chemical binding of insulin by muscle, lactating mammary gland, and adipose tissue has been well established in a number of laboratories. This demonstration of insulin binding by the rat diaphragm is simple. A normal diaphragm is dipped momentarily into a solution of radioactive insulin. The bound insulin may now be measured in two ways: (1) its radioactivity; (2) when the diaphragm is equilibrated in glucose. The customary significant increase in glycogen synthesis and synthesis and glucose uptake due to the presence of insulin is always demonstrated as contrasted to the normal untreated diaphragm. The binding of insulin by tissues is a very rapid

process. As little as 10 seconds of exposure of the diaphragm to a solution of insulin suffices. The bound insulin shows the usual endocrine interrelations, namely, decrease of activity in the diabetic animal, marked increase of activity in the hypophysectomized animal, depression of activity following prior injection of growth hormone plus cortisone, etc.

### METABOLIC ADAPTATION BY INSULIN

Recently attention has been focused upon a phenomenon which might be called metabolic adaptation brought about by insulin. By adaptation I mean that in the presence of insulin a change in the function of enzymatic systems of tissues occurs which requires considerable time for full development. This phenomenon has been well summarized by Hastings and his co-workers[20] who studied it at considerable length. Their results in the case of the diabetic rat are shown in Figure 6. Using the diaphragm as an example of muscle, they showed that the response of injected insulin was immediate. A minute or two or less after the injection into the intact rat there is a significant increase of glucose uptake and glycogen synthesis when the isolated diaphragm is equilibrated *in vitro*. Liver slices, however, from the same rat responded to the injected insulin very slowly so that periods of 6 to 24 hours were required for full restoration from the diabetic to the normal status. As measures of response to insulin Hastings quoted, among other effects, the total phosphorylation of glucose, the incorporation of fructose, pyruvate, or acetate into higher fatty acids. The responses in the case of the liver are strikingly different from those of the muscle. Insulin reaction in the diaphragm is essentially instantaneous and appears to be concerned with the transport of glucose. In contrast, insulin action in the liver is slow and in the diabetic can only be demonstrated by injection into the intact animal. A reasonable hypothesis would be to assume that, by some mechanism which at present is not known, insulin permits the liver slowly to complete an enzyme system which in the absence of insulin has lost its function in whole or in part. The adaptation requires perhaps synthesis of new proteins or the formation of co-factors. These reactions take time and further suggest the possibility of diverse types of insulin action in the liver

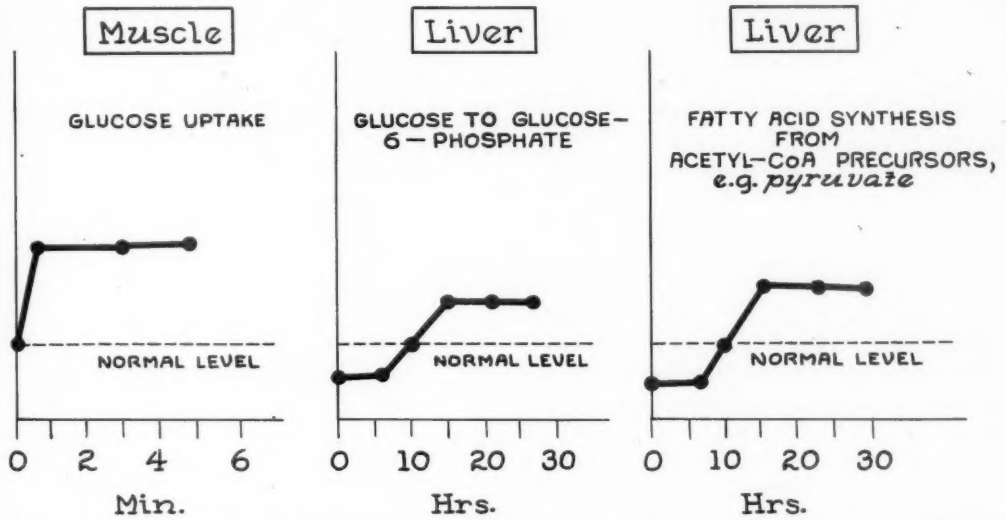



Fig. 6. Sequence of events following injection of insulin into diabetic rats. Data of Renold, Hastings, Nesbett, and Ashmore, 1955.

affecting glucose or fatty acid metabolism.

On the basis of these considerations, it is possible to formulate, as a working hypothesis, what might be called a unified concept of insulin action. I show this in Table VI. Four

TABLE VI

Unified Concept of Insulin Action. Date of Stadie, 1956

| Tissue | Mechanism of action | Site of action | Chief metabolic effects |
|---|---|---|---|
| Muscle | Combination | Cell surfaces | Glucose transport *et seq.* |
| Liver | Adaption | Enzyme systems; contra-pituitary | Glucose oxidation, etc., fatty acid synthesis; oxidative phosphorylation (?) |
| Adipose | Adaption | Enzyme systems | Glucose oxidation, etc., fatty acid synthesis |
| Brain | No demonstrable action | | |
| Other | Insufficient data | | |

columns are considered: (1) tissue; (2) mechanism of action; (3) site of action; and (4) chief metabolic effects. In muscle, the mechanism of action may be considered to be a combination of insulin with the muscle and the site of the action perhaps upon cell surfaces. The main metabolic effects would be an increase of glucose transport which of course would accelerate phosphorylation and therefore further reactions. In the liver adaptation is necessary, time is required, the enzyme systems are under the contra control of pituitary factors, and the chief metabolic reactions involved are glucose oxidation, fatty acid synthesis, oxidative phosphorylation and perhaps others. In adipose tissue, the situation is about the same as that outlined for the liver. In the brain, as many experimenters have found, there is no demonstrable action of insulin and in other tissues the data available are insufficient to make discussion profitable.

This unified concept of insulin action need not be complete nor even free of errors so long as it has some meaning to the experimenter and is provocative. If the concept accomplishes a little in this direction, it will have served its purpose.

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# Effects of Growth Hormone on Protein and Carbohydrate Metabolism

*By* JANE A. RUSSELL, PH.D.*

THE EFFECT of growth hormone on protein and carbohydrate metabolism has by now become so extensive a subject as to preclude any complete review within the space allotted. Instead, I should like to present the evidence, some old, some new, on a few selected aspects of the subject which may be of particular interest to this conference on hormones and nutrition. In doing so, I may also be able to summarize in outline one point of view, a possible hypothesis, concerning the normal physiologic role of growth hormone in metabolism.

The term *growth hormone* in this connection is to be understood to include also the phrase "and/or other closely related pituitary factors;" for although as yet no convincing evidence has been presented for the existence of separable diabetogenic or other metabolic factors from the pituitary, some effects of certain preparations have appeared not to be attributable entirely to growth hormone alone. These effects, which include the ketogenic and the hypoglycemic responses among others, may perhaps be the result of the combined actions of growth hormone with known factors such as the adrenotrophic or thyrotrophic hormone, or of growth hormone with unknown factors, or even entirely of some as yet unidentified principals in the pituitary gland. Since, however, the most highly purified growth hormone preparations available have been found to reproduce nearly all of the effects of pituitary on metabolism, I should like, for convenience, to use the term growth hormone in an inclusive sense.

You are all familiar with the fact that the pituitary growth hormone is necessary for growth to normal size in young animals, and probably you are also acquainted with much of the evidence which indicates that growth hormone (or the related factors just mentioned) may give in certain circumstances effects which are described as diabetogenic, ketogenic, glycostatic, contra-insulin, adipokinetic, and the like. The normal role of growth hormone cannot be purely passive, simply allowing growth to occur in accordance with genetic and nutritional determinants; for when an excess of growth hormone is given either to young or to older animals, it promotes increased growth and nitrogen retention beyond the normal limits expected in a given set of conditions. This growth is orderly and it occurs in most, or more probably all, organs or tissues, although not necessarily to the same extent in every case. Hence, growth hormone must be able in some manner to take an active part in determining the nitrogen balance in the animal body, and there appears no reason why this effect should be limited to the young growing animal. The question is, then, what is the normal physiologic role of growth hormone with respect to retention of nitrogen as tissue protein, and in this sense of growth, in the normal adult animal?

With respect to the so-called diabetogenic effects, as Houssay remarked long ago[1] the normal role of the pituitary in metabolism cannot be to produce diabetes. This and similar pathologic effects must be considered as manifestations either of a large excess of the hormone or of a moderate excess acting under

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conditions which exaggerate the results of its action. What we must look for here, and have yet to find for certain, is some function for the pituitary metabolic hormone which can be reasonably considered to contribute to the maintenance of the normal animal economy of carbohydrate and fat.

The hypothesis which I mentioned earlier is an attempt to answer these questions concerning the normal role of growth hormone.

## HYPOPHYSECTOMY

Let us first turn our attention to the question of nitrogen retention and nitrogen balance. Nitrogen retention in the tissues in the form of protein, if not synonymous with growth, is obviously an essential and integral part of this process; and administered growth hormone has been demonstrated repeatedly to induce or to augment a positive nitrogen balance in this form. This fact raises the important question, is there a mirror or negative image of this effect? If growth hormone may be considered to have a positive function in the intact grown animal, then when the source of growth hormone is removed, we should expect to see the development of a negative nitrogen balance. The experimental data on this point have been in the past somewhat contradictory. It is known that the hypophysectomized animal may or may not lose body tissue in the immediate postoperative period, depending mainly on the age of the animal (very young animals may continue to grow a little for a time) and on the amount of food ingested in this period. Usually, the voluntary food intake drops precipitously, and this, of course, is accompanied by loss of body tissue and loss of nitrogen. Later, the body weight of the animal tends to become stable, perhaps to be maintained indefinitely, and in this case nitrogen balance is established. During the latter period, the endogenous or minimal nitrogen catabolism and the fasting nitrogen excretion have been reported to be less than normal[2,3] instead of excessive, as might be predicted for the absence of growth hormone. However, it is easily seen that in this circumstance the low rate of "endogenous" nitrogen catabolism may well be a secondary occurrence. There is first the fact that the food intake will probably have been low for some time before the observations were made. Then, it must be remembered that by this time the animal will suffer from deficiency of both thyroid and adrenocortical hormones, and both thyroidectomy and adrenalectomy have been reported to be followed by similar reduction in endogenous nitrogen catabolism. Hence, these observations are not critical as to the character of nitrogen catabolism in the absence of growth hormone alone.

However, for the period just following operation some observations do indicate differences in the expected direction. The oldest of these is the work,[4] summarized in Table I.

TABLE I

Composition of Hypophysectomized and Normal Rats Pair-Fed* during 33 Days Following Operation (from Lee and Ayres[4])

| | Composition in g/100 g initial body weight | | | |
|---|---|---|---|---|
| | Body wt. | Protein | Water | Fat |
| Initial | 100.0 | 17.8 | 64.0 | 13.1 |
| Final | | | | |
| Normal | 81.2 | 18.2 | 54.0 | 5.3 |
| Hypophysectomized | 74.0 | 14.3 | 45.7 | 9.3 |

* Food intake equaled the amount eaten voluntarily by the hypophysectomized animals. Analyses from 16 rats per group.

Analyzing the bodies of rats which were either normal, hypophysectomized, or normal but pair-fed with the animals operated upon, it was found that whereas the normal animals lost no nitrogen on this regime, the hypophysectomized rats lost about one-fifth of their body nitrogen during the postoperative period. The normal rats of course lost fat, in this case twice as much as the operated animals. Thus, at least when the food intake was restricted, the animal was unable to maintain nitrogen balance when growth hormone was lacking.

Other data[5] compare the effects of restricted food intake with those of force-feeding (Table II). In this case, the control animals were underfed to the extent that their weight loss equalled that of the hypophysectomized group

TABLE II

Changes in Body Composition of Hypophysectomized Rats during Post-Operative Period of 3 Weeks (from Levin[5])

| | Gram per 100 *g* initial body weight | | | | |
|---|---|---|---|---|---|
| | No. | Body weight | Water | Protein | Fat |
| 1. Initial Composition | 28 | 100 | 62.4 | 21.0 | 11.6 |
| 2A. Normal, Restricted Food Intake | 11 | −27.1 | −14.1 | −3.9 | − 8.5 |
| 2B. Hypophysectomized, ad lib. | 14 | −27.6 | −17.0 | −5.3 | − 3.9 |
| 3A. Normal, Force-Fed | 10 | + 8.4 | + 1.7 | 0.0 | + 6.6 |
| 3B. Hypophysectomized, Force-Fed | 12 | + 8.8 | − 4.4 | −1.4 | +14.5 |
| Av. Standard Error | | | ± 0.9 | ±0.4 | ± 1.0 |

eating *ad libitum*. Hence the food intake of the former was probably more severely restricted than that of the operated subjects.

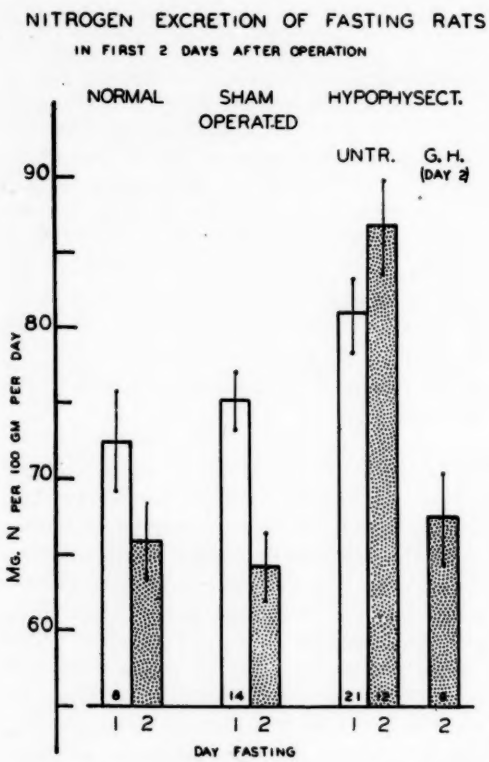


Fig. 1. The acute effect of hypophysectomy on fasting nitrogen catabolism. The animals were fasted from the time of operation. Growth hormone, 0.5 mg per 100 g body weight, was given in two doses during the second day postoperative. Vertical lines indicate standard errors; small numbers at base of columns are numbers of observations per group.

Nevertheless, the loss of nitrogen was again much greater in the absence of the pituitary. On the other hand, if the normal and hypophysectomized rats both were force-fed the same quantity of food (equal to the normal intake), the loss of nitrogen by the operated animals was reduced to a barely significant amount. Here we see that although the recently hypophysectomized animal is unable to conserve nitrogen to a normal degree when the amount of nitrogen taken in is limited, it may be able to maintain nitrogen balance, or nearly so, when the amount of food ingested is adequate.

The experiments just described were chronic in nature and do not necessarily indicate what might be expected to happen with deprivation of protein over a more limited period of time. Past observations of an acute type have been made only on animals which previously had undergone variable periods of reduced food intake and whose metabolic rates had been reduced by thyroid atrophy, as indicated above. For this reason, we have repeated and extended some observations, made originally by Long and Fry but not published as far as I know, on the effects of hypophysectomy on the nitrogen excretion of rats fasted from the time of operation. The results are summarized in Figure 1. On the day following operation, we have found that the nitrogen excretion of hypophysectomized rats tends to be increased as compared with that of untreated fasting rats, but so is that of sham-operated controls; and the differences among these groups are at most only marginally significant. However, on the second day the behavior of the hypophysectomized rats was quite different: where

the nitrogen excretion of the normal rats usually fell and that of the sham-operated animals almost always was considerably lower on the second day than on the first, that of the animals lacking growth hormone tended to increase still further, so that a large and highly significant difference developed. If, however, growth hormone (0.5 mg per 100 g) was given during the second day, the nitrogen loss was reduced immediately to control levels. Thus, growth hormone appears to be necessary for a normal degree of nitrogen conservation during the acute deprivation of food over a limited period of time, as well as when the food intake is chronically restricted.

### NITROGEN RETENTION

The magnitude of the effect of exogenous growth hormone has generally been found to be a function of the quality and of the quantity of the protein sources available. Gordan and Bennett and co-workers[6] have shown quite clearly that in rats on a diet containing only 6 per cent casein, the amount of methionine available strictly limits the amount of nitrogen which will be retained when growth hormone is given. A complete assortment of amino acids would then appear to be essential, although admittedly this has not been established for the whole range of amino acids.

Further, in fasting animals which are otherwise normal it is difficult to induce nitrogen retention with growth hormone, although it can be done to a limited degree if larger amounts of the hormone are given.[7,8] In Table III, taken from the work of Bennett, Gordan, and co-workers, the effects of purified growth hormone on nitrogen retention are compared in fasting and in fed rats. A large dose of growth hormone induced only barely significant retention of nitrogen in rats fasted for two or three days, whereas in other experiments in fed rats the nitrogen excretion could be reduced by nearly half with doses of similar order. That the limitation here is in the supply of amino acids, rather than in that of the non-nitrogenous constituents of the diet, is indicated by studies of the effects of growth hormone on rats of urea formation as measured in nephrectomized rats. In this work, which will be discussed further a little later, the effect of the hormone on nitrogen "retention" was fully evident in otherwise fasting rats when a mixture of amino acids only was supplied.

TABLE III

Effect of Growth Hormone on Nitrogen Excretion of Fed or of Fasting Rats

| | Mg N/100 g/day | | |
|---|---|---|---|
| | Fasting* | Fed † 6% casein + methionine | Fed† 18% casein |
| Untreated | (45) 50 ± 2 | (10) 87 ± 2 | (10) 206 ± 2 |
| Growth Hormone | (14) 43 ± 2 | (10) 48 ± 2 | (10) 114 ± 2 |

* Days 2 and 3 of fasting period, 2 mg G. H./day (Bennett *et al.*, 1948[3]).

† Five-day period, 1 mg G. H./day (Gordan, *et al.*, 1948[10]).

Finally, Gordan, *et al.*[9] have shown that as the protein intake is increased, the nitrogen retention obtained with a given dose of hormone increases proportionately until a maximum effect is reached at quite high intakes.

These results taken together have an important theoretic bearing on the nature of growth hormone action on nitrogen metabolism. They must probably mean that growth hormone enhances the rate of some phase of protein synthesis; for if the hormone were to act otherwise, by inhibiting either the breakdown of protein to amino acids or the catabolism of amino acids, then its effects on nitrogen retention should be equally evident regardless of the kind or quantity of amino acids available.

A point in this connection which is often overlooked is that the amino acids necessary for the formation and deposition of protein need not always be of exogenous origin only, but conceivably might come also from endogenous sources. I believe that we have already seen an example of this in the fasting hypophysectomized rats described earlier, for here growth hormone brought about a large degree of nitrogen retention in the absence of exogenous sources but in presence of what otherwise would have been an excessive rate of protein loss. Another example is provided by the work of Volk and Lazarus,[10] whose data are presented in Table IV. In this experiment, a modest dose of growth hormone was without

significant effect in the fasting normal animal but induced a marked degree of nitrogen retention when the rate of catabolism of protein had been much increased during dosage with phlorizin. When the animals were adrenalecto-

TABLE IV

Effect of Growth Hormone on Nitrogen Excretion in Fasting Rats (from Volk and Lazarus[10])

| | Mg N/100 g/day | |
|---|---|---|
| | Fasting | Fasting with Phlorizin |
| Normal rats | (12) 60 ± 2 | (24) 116 ± 4 |
| Normal + Growth Hormone* | (14) 54 ± 3 | (12) 70 ± 5 |
| Adrenalect. rats | (12) 55 ± 2 | (22) 50 ± 4 |
| Adrex. + Growth Hormone* | (22) 45 ± 4 | (21) 52 ± 2 |

* 0.1 mg equivalent per 100 g per day, x 3.

mized, this effect of phlorizin was lacking, as is well known, and so was the effect of growth hormone. If it is true that growth hormone affects primarily the synthetic aspect of nitrogen metabolism, then in both of the cases just described the source of the nitrogen retained must have been the amino acids coming from body tissues. In the one case, these were in relative excess because of defective synthesis; in the other, they were increased by mechanisms which are not known but which evidently are dependent on the presence of adrenocortical hormones.

Thus, since a mixture of amino acids coming from body tissues will always be available in some degree, it is not precisely true to say that growth hormone is without effect in fasting animals. Rather, our ability to detect the effect of a given dose of hormone will depend on the net rate of protein catabolism relative to the sensitivity of the methods employed.

### DIETARY CARBOHYDRATE

Another usual requirement for nitrogen balance in normal animals, besides adequate protein intake, is the provision of moderate amounts of carbohydrate. This is readily demonstrated either by substituting fat for carbohydrate in the diet or by separating in time of day the ingestion of carbohydrate from that of the protein. In either case, a negative nitrogen balance develops promptly. As Munro[11] has pointed out, however, this requirement is probably only temporary, lasting for several days or longer but then disappearing as the animal appears to adjust to the new regime. The mechanism of this adjustment becomes an interesting question, about which virtually nothing is known at present.

Considering that growth hormone is most effective in fed animals, perhaps it might be expected that some carbohydrate would be necessary for the action of the hormone on nitrogen balance, or that the effect could be augmented by extra carbohydrate. From present indications, however, it appears that dietary carbohydrate is not essential for nitrogen retention under the action of growth hormone and that administered hormone may even be able to compensate entirely for a deficiency of carbohydrate, thus allowing nitrogen balance to occur when this would not otherwise be possible. This was first indicated by the work of Cuthbertson, Webster, and Young in 1941.[12] When the carbohydrate and protein of the diet of rats were separated in time, the concurrent injection of a crude pituitary growth hormone preparation not only prevented the nitrogen loss otherwise seen but even induced a considerable positive nitrogen balance. This experiment needs now to be repeated with purified growth hormone, but there seems no reason to doubt that the results would be similar to those just described.

Perhaps to be placed in parallel with this experiment are observations, summarized by Munro,[11] indicating that dietary carbohydrate is not required for growth in young animals. Although, as Munro remarked, one could wish for more conclusive evidence on this point, it is tempting to suppose that the animal's own growth hormone could be instrumental here, and that perhaps, too, the adjustment to lack of carbohydrate by the adult could be a function of its pituitary secretion.

### AMINO ACID METABOLISM

Further evidence on the role of dietary constituents in the action of growth hormone

has been derived from some observations on the rates of urea formation in nephrectomized rats.[13–15] In this work, the increase in blood urea is measured during a period of one or two hours. In the absence of exogenous nitrogen, the rate of protein degradation is low and little affected by the prior administration of growth hormone. When mixed amino acids in the form of casein hydrolysate are furnished in moderate amount, the rate of urea formation is of course much increased; but this increase can be depressed to a highly significant degree if growth hormone is given an hour or two before the amino acids. Changes in the rate of urea formation in these circumstances probably may be considered as inverse reflections of alterations in the rate of synthesis of protein or catabolized when growth hormone was given shortly before the amino acids. When glucose was given to the fasting animal, nitrogen "retention" occurred, as might have been expected, but here no further effect of growth hormone was evident. In these acute conditions, then, it appears that not only is exogenous carbohydrate not required for growth hormone action, but that the effect of the hormone is best demonstrated when fat is forming the principal part of the metabolic mixture.

## COMMENT

The suggestions that one may derive from the data just outlined are, first, that growth hormone is required for normal nitrogen

TABLE V

Urea Formation from Casein Hydrolysate* in Nephrectomized Rats

| | Increase in blood urea nitrogen in 2 hours mg % N | | |
|---|---|---|---|
| | Control | Growth hormone | G. H. Effect |
| 1. Fasted 24 hours | (10)† 22.2 | (7) 14.5 | $-7.7 \pm 0.7$ |
| 2. Not fasted, carbohydrate diet | (10) 19.0 | (10) 17.5 | $-1.5 \pm 1.0$ |
| 3. Not fasted, fat diet 4 to 6 days | (8) 15.3 | (9) 10.6 | $-4.7 \pm 1.0$ |
| 4. Refed glucose 5 hours (after 18 hour fast) | (6) 16.3 | (7) 17.2 | $+0.9 \pm 0.6$ |
| 5. Refed oil emulsion 5 hours (after 18 hour fast) | (6) 21.3 | (6) 15.2 | $-6.1 \pm 2.0$ |

* Parenamine®, 13.5 mg N/100 g body weight, intravenously, 3 to 4 hours after operation.

† = Number of observations.

peptides, rather than as expressions of direct effects on amino acid catabolism.

As noted previously, the effect of growth hormone on nitrogen "retention" in these experiments was fully evident in rats which had first been fasted and then given mixed amino acids only. When this experiment was repeated in rats which had been ingesting their usual diet high in carbohydrate but had not been fasted, we found, somewhat to our surprise, that no significant effect of growth hormone could be obtained. However, as shown in Table V, when the animals had been given for several days a diet low in carbohydrate but high in fat, with moderate amounts of protein, less urea was formed from the amino acid mixture alone. Still less nitrogen was retention in the grown subject, at least when the food intake is limited, and secondly, that an excess of growth hormone can bring about nitrogen retention from exogenous or endogenous sources of amino acids in the absence of dietary carbohydrate. Putting these two ideas together suggests a third possibility: that growth hormone has a particular function in enabling nitrogen conservation whenever the supplies of protein or of carbohydrate are restricted, that is, between meals, as it were. If this were true, one might imagine that either the activity of growth hormone or its rate of secretion could be increased to assist in the metabolic adjustments which the animal must make when the absence of food threatens its economy.

### CARBOHYDRATE METABOLISM

We now come to the question of a role for growth hormone in carbohydrate metabolism. The observations on the diabetogenic activity of pituitary extracts and on the Houssay effect, important as they have been in demonstrating the participation of hypophysial hormones in metabolism, have necessarily been somewhat gross in character. From what is now a rather large body of evidence on these points, we still do not know just what the pituitary factor does to the carbohydrate metabolism of the animal, besides sometimes inducing glycosuria, nor do we understand entirely what happens to the carbohydrate metabolism of the hypophysectomized animal.

Of the facts which are known in this connection, only a few may be cited here. One of the alterations which has been observed repeatedly in several different ways is that in the absence of the hypophysis the animal is unable to conserve carbohydrate during fasting. The R. Q. is high, in several species. Not only do the blood glucose and liver glycogen fall much more rapidly than in normal fasting animals, but the muscle glycogen, the major site of carbohydrate storage because of its mass, also tends to be diminished. When carbohydrate is fed, it too disappears at a rapid rate. In Figure 2 are shown some early data on the carbohydrates of the tissues in hypophysectomized and normal rats first fasted and then fed. Although the disappearance of the starch from the gastrointestinal tract was somewhat slower in the operated animals, it was nearly all gone in the time allowed here for absorption. Yet, the glycogen stores and blood glucose could not be maintained as they were in the normal animals, even when twice as much carbohydrate had been fed to the hypophysectomized rats. It is difficult to explain these observations as the results of other than an

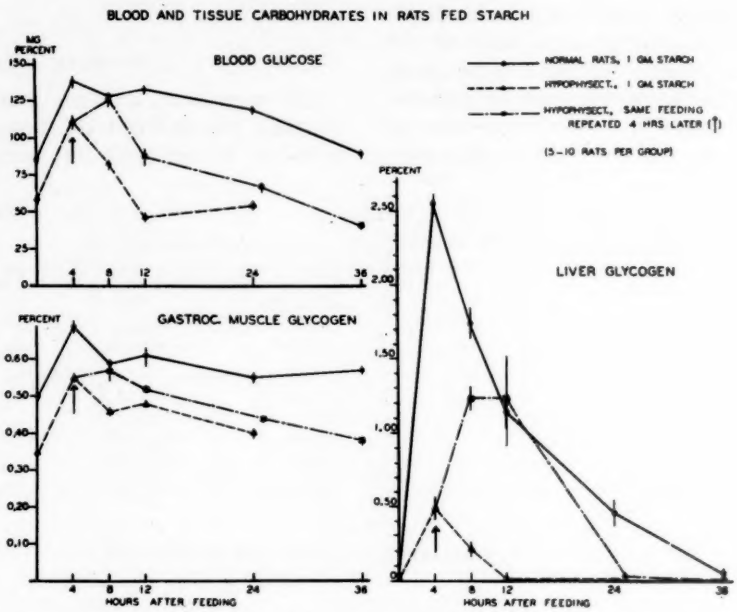


Fig. 2. The disappearance of fed carbohydrate in hypophysectomized and normal rats (data from Russell and Bennett[24]). The animals were first fasted 20 hours and then fed by tube a suspension of corn starch. In one group of hypophysectomized rats a second similar feeding was given 4 hours after the first, as indicated by the arrow. The vertical line at each point indicates the standard error.

abnormally rapid rate of carbohydrate utilization.

When growth hormone is given acutely either to normal or to hypophysectomized animals, the direction of effects is reversed from that just seen. The R. Q. is diminished and carbohydrate is conserved, mainly as muscle glycogen.[16,17,18] In some species, after a few days the blood sugar may be raised and the glucose tolerance reduced, although in the rat and perhaps some other species excess cortical hormone appears to be required for the exhibition of the frankly diabetic state.[19,20] In a manner not understood, the hormone must be able to restrain the utilization of carbohydrate. Also, it should be noted that since the metabolic rate is not reduced, there must be a reciprocal relationship between the utilization of carbohydrate and that of fat; and, in fact, the latter fuel does appear to be used less extensively in hypophysectomized animals during fasting or in the postabsorptive period, and to be transported and catabolized to a greater extent than normally in the presence of excess pituitary hormones.

## CARDIAC GLYCOGEN

These few observations on carbohydrate metabolism, most of which are well known, have been indicated only briefly as a background to the next topic, some recent work on the effects of growth hormone on the glycogen of the heart. The heart differs from skeletal muscle in that it is working continually, yet always maintains a rather high concentration of glycogen. Because the glycogen content remains fairly constant, even when the heart is withdrawing glucose or other materials from the blood at a rapid rate, the cardiac glycogen has been thought to act only as an emergency substrate. However, although it does constitute a reserve energy source in this sense, the glycogen of the heart is not immobile. The mechanisms of carbohydrate utilization in the heart seem to be practically identical with those of other muscles, and when the heart must work under anaerobic conditions, the cardiac glycogen disappears with extreme rapidity. Also, in the course of some recent work in our laboratory, it has been found that carbon-labelled glucose from the blood is incorporated into cardiac glycogen to a greater extent than it is into skeletal muscle.[21] This indicates probably that the turnover of glycogen in the working heart muscle is at least as rapid as in other organs. Thus, the glycogen of the heart might be expected to be responsive to hormonal influences governing the uptake of glucose and its utilization in a fashion similar to, if not necessarily identical with, that of skeletal muscle glycogen.

However, cardiac muscle glycogen has been known for many years to display one anomaly: during fasting, and also in diabetes mellitus, the glycogen of the heart is increased, instead of being depleted to a greater or lesser degree as it is in other organs. This curious circumstance has been observed in a variety of species. Some data on the changes seen in the rat heart are shown in Figure 3, taken from Adrouny and Russell.[22] In this series, the maximum glycogen content was attained after 36 to 48 hours of fasting and was maintained thereafter for many days. It may be noted that these

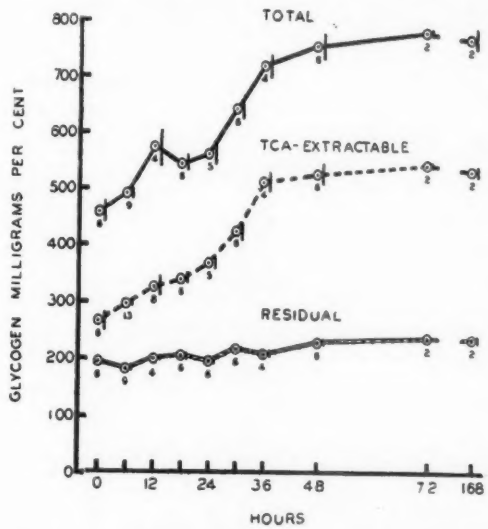


Fig. 3. The effect of fasting on the glycogen of the heart in rats (from Adrouny and Russell[22]). The concentration of glycogen in the whole heart is related to time after withdrawal of food. Vertical lines indicate standard errors; small figures, the number of observations at each point.

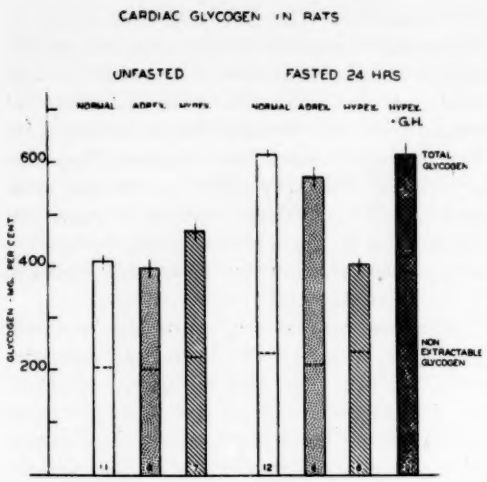


Fig. 4. Effect of fasting on the cardiac glycogen of rats with and without pituitary growth hormone (data from Russell and Bloom[23]). *Adrex.* indicates adrenalectomized rats maintained with saline drinking fluid. *Hypex.* indicates hypophysectomized rats. Growth hormone, 0.5 mg per 100 g body weight, was given in 3 doses during the 24-hour fasting period.

changes occurred almost entirely in the fraction of glycogen which was extractable with trichloroacetic acid, whereas the non-extractable or protein-bound fraction remained virtually constant throughout. This has been true in most of the other experiments of this general nature which we have done. The meaning of this dichotomy of the glycogen of animal tissues is not yet known.

The increase in cardiac glycogen which occurs normally during fasting has now been found in the rat to require either the presence of the hypophysis or the administration of growth hormone during the fasting period.[23] This is illustrated in Figure 4. Adrenalectomy did not prevent the occurrence of the normal increase in cardiac glycogen during fasting, nor did the administration of cortical hormone reproduce the effect of growth hormone. From more recent work[22] the minimal dose of bovine growth hormone which will affect the cardiac glycogen of the rat is of the order of 30 μg per 100 g when given in two or three doses

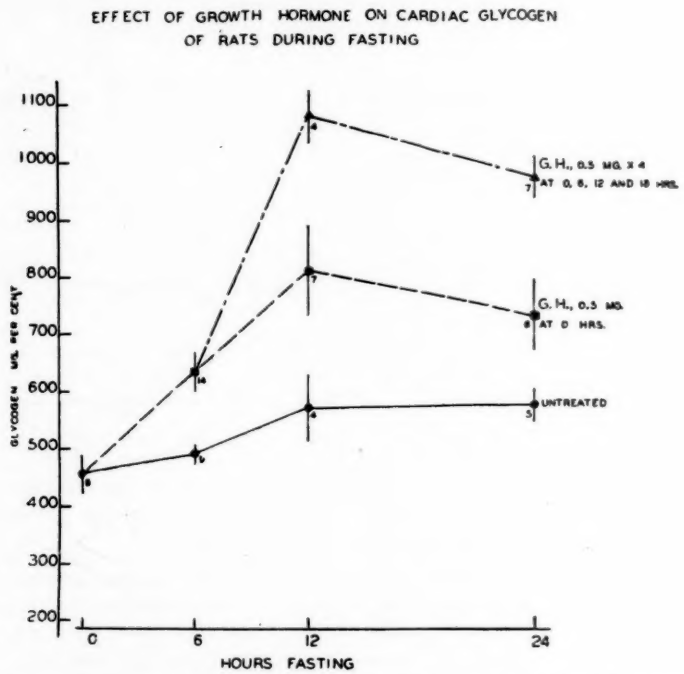


Fig. 5. Increase in cardiac glycogen of normal rats given growth hormone during fasting (data from Adrouny and Russell[22]).

during the 24-hour fasting period, or about the same as that required to induce a positive nitrogen balance.

If growth hormone is an active physiologic agent responsible in some manner for the normal increase in cardiac glycogen in fasting, rather than only a passive or permissive factor, then with an excess of the hormone it should be possible to alter the glycogen of the heart of normal animals. This expectation has been verified, as shown in Figure 5. The lower line here indicates the rising concentration of glycogen in the heart in normal animals during the first 24 hours of fasting. When growth hormone in rather large dosage was given at the start of the fasting period, the increase in cardiac glycogen was greatly hastened, and with repeated dosage extremely high concentrations of glycogen could be obtained. Smaller doses of the hormone also were effective in these circumstances, detectable increases in cardiac glycogen being obtained at six hours with doses of the order of 50 $\mu$g per 100 g body weight.

The mechanism by which growth hormone brings about the increase in cardiac glycogen is not known. The effect is obviously not mediated by hyperglycemia, for the blood glucose falls during fasting, and growth hormone in these experiments had no effect on the blood glucose except perhaps to lower it slightly. In fasting rats, the incorporation of labeled blood glucose into the cardiac glycogen is slower than it is in the hearts of fed rats, so that it is doubtful whether the increase of glycogen during fasting is the result of an increased rate of transfer of blood glucose into the cardiac muscle.[21] Although the increase in glycogen conceivably could be the result of gluconeogenesis, this does not seem likely from what is known at present of the metabolism of the heart muscle. We are left, then, with the possibility that the higher concentrations of glycogen simply reflect diminished rates of utilization of carbohydrate. The latter concept agrees more closely than any other with what is known of the action of pituitary factors in other parts of the body, and so until further evidence is obtained, it may be considered the most probable course.

Whatever the mechanism of this effect, the close parallelism between the alterations in cardiac glycogen brought about by fasting and by growth hormone, the necessity of the pituitary for the fasting effect, and its exaggeration by exogenous growth hormone, all suggest that the hormone is instrumental normally in this adjustment to fasting. In this case, either the activity of growth hormone or its rate of secretion must be increased during fasting.

A further question then arises: what is the essential difference between the fasting and fed states which would control the action of growth hormone? A partial answer to this question has been sought by feeding the three major foodstuffs separately to previously fasting rats. The results of such an experiment have been summarized in Figure 6. In

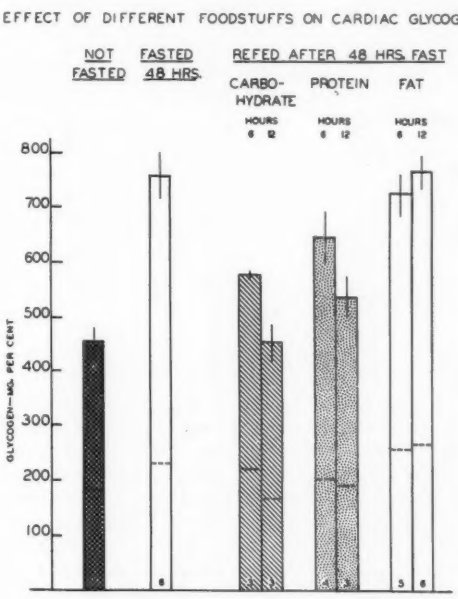


Fig. 6. Cardiac glycogen in rats 6 and 12 hours following refeeding after a preliminary fasting period (data from Adrouny and Russell[22]). Carbohydrate fed was 2 g of cornstarch in aqueous suspension; the protein was 1.5 g of casein; and the fat was 1 or 2 ml of olive oil in stable emulsion.

animals first fasted 48 hours, approximately equimolar amounts of starch, casein, or an oil emulsion were given. Here, the feeding of

carbohydrate was followed promptly by loss of cardiac glycogen, and so was the ingestion of protein, although perhaps somewhat more slowly. Fat, on the other hand, had no effect at all. In these experiments, the liver glycogen of the protein-fed animals was only slightly lower than that of the rats given carbohydrate, whereas that of the fat-fed group was not much

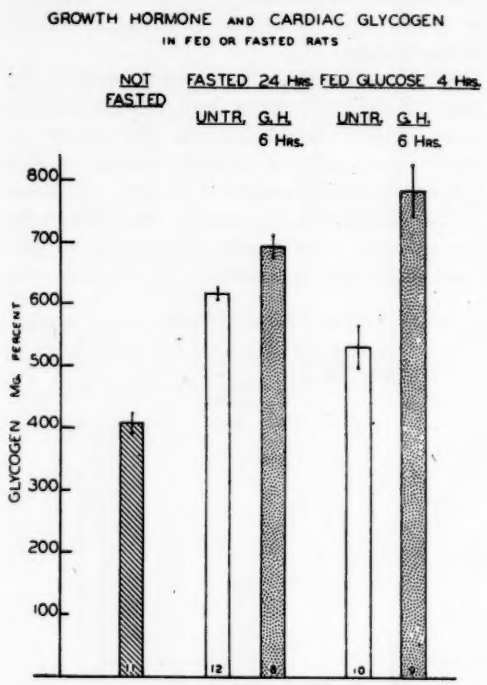


Fig. 7. Comparison of effects of growth hormone on cardiac glycogen in fasting rats and in those fed glucose (data from Russell and Bloom[23]). Growth hormone, 1 mg per 100 g body weight, was given after 18 hours of fasting, and glucose (1 g) was given at 20 hours, with analyses at 24 hours in all groups except unfasted controls.

different from that of fasting rats. By inference, then, it seems possible that the cue or trigger for the suppression of growth hormone activity could be provided by an excess of carbohydrate or of one of its metabolic products. Alternatively, however, one could suggest that some metabolite of fat exerted a positive effect either on the cardiac glycogen or on the activity of growth hormone. It is going to be difficult to separate the parts played by agents which are related in the reciprocal fashion of fat and carbohydrate metabolism, but I believe an experimental approach is feasible.

Finally, the argument may be taken one further step. It has been shown that feeding of carbohydrate suppresses cardiac glycogen and that continued fasting augments it, but it has not been indicated whether the fed or fasting state may affect differentially the action of exogenous growth hormone on cardiac glycogen. One set of observations on this point is shown in Figure 7. When growth hormone was given to the fasting animals, whose cardiac glycogen was already somewhat elevated, a small but significant further increase occurred. When glucose was given alone, the usual diminution in cardiac glycogen was seen four hours later. If, however, growth hormone was given first and then glucose, not only was the action of the glucose completely overcome but the cardiac glycogen may have been increased to an even greater degree than it was in the fasted animals. In other experiments also, of like nature but employing different periods of fasting, feeding, and treatment, the results have been qualitatively similar.[22] Hence, no evidence has been obtained to indicate that the feeding of carbohydrate interferes with the action of exogenous hormone on cardiac glycogen or that fasting exaggerates it. Until more critical information is at hand, it appears that the fasting state must affect the rate of secretion of growth hormone, rather than its activity *per se*.

## CONCLUSION

Now we may try to combine, in theory at least, the effects of growth hormone on nitrogen metabolism with some of those on the metabolism of carbohydrate. As was indicated earlier, there is reason to believe that growth hormone is instrumental in the retention of nitrogen in body tissues, particularly when the supply of exogenous protein or of carbohydrate is restricted. In respect to carbohydrate also, we have seen that growth hormone (or the hypothetical closely related pituitary substance), is a conservative factor. Normally, whenever the supply of carbohydrate becomes limited its oxidation too is diminished, and

that of fat is increased. We may suppose then that one of the functions of the pituitary is to facilitate this shift in the substrate of metabolism. What little is known of the role of the hypophysial hormones in fat metabolism is in general agreement with this hypothesis, although it must be admitted that the details of the action of growth hormone in this respect are not yet settled. If the action of growth hormone on cardiac glycogen may be taken as in any way representative of the role of this hormone in carbohydrate metabolism elsewhere in the body, then we have the additional suggestion from the data just discussed that the rate of secretion of the pituitary hormone may not be constant but instead can be augmented when it is needed in order to promote the conservation of carbohydrate. This could be expected to be true also of the role of the hormone in nitrogen metabolism, although we have no critical evidence on this point.

This has been to a large extent a hypothetical construct, full of suppositions which may or may not be supported by further investigations. However, I believe that the hypothesis is consistent with the facts as far as they are known and that it is susceptible to experiment. Meanwhile, it has led me to one final supposition, that growth hormone, or its companions, may soon be added to the lengthening list of hormones which are instrumental in the day-to-day or hour-to-hour adjustments of the animal economy to the impositions of external circumstance.

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# The Influence of the Endocrine Glands on Fatty Acid and Ketone Body Metabolism*

*By* FRANK L. ENGEL, M. D.†

THE PHENOMENON of ketosis has intrigued physicians and biochemists ever since the ketone bodies, acetoacetic acid, beta-hydroxybutyric acid and acetone were identified almost 100 years ago in the urine of patients with diabetic acidosis.[1] The decade beginning in 1930 marked the opening of a period of intense interest in the role of the hormones in ketosis, sparked by the almost simultaneous discovery and investigation of the ketogenic activity of certain pituitary extracts by Burn and Ling,[2] Anselmino and Hoffman,[3,4,5] Black, Collip and Thomson,[6] and Houssay and Rietti.[7–10] Especially noteworthy during this period was the work in the laboratories of Houssay,[11–13] Long,[14–16] Mirsky,[17–20] Somogyi,[21,22] and Stadie.[23] Progress in this field was impeded to some degree, however, by methodologic difficulties, by lack of availability of purified hormones and by large gaps in our knowledge concerning the details of the metabolic processes involved in fat catabolism and anabolism and in ketogenesis itself. Thanks to the brilliant work of biochemists in the past decade these major blocks to progress have been largely overcome, a fact which has stimulated us to reinvestigate certain aspects of the problem of the influence of the endocrines on fatty acid and ketone body metabolism.

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## METABOLIC CONSIDERATIONS

The elucidation of the pathways of fat metabolism was initiated by the demonstration of fatty acid catabolism in a cell-free system by Leloir and Munoz in 1939[24] and brought to fruition by the work of Lehninger,[25–27] Lipmann,[28] Lynen,[29] Ochoa,[30] Green,[31] and others who rapidly worked out the details of the metabolic steps and enzyme systems involved in fat synthesis and degradation. While there are still many biochemical questions to be answered, the status of the field is now such as to allow for a much more rational interpretation of the phenomenon of ketosis and of the influence of hormones thereon.

The chief ketone body is acetoacetic acid; from this is derived beta-hydroxybutyric acid by reduction, and acetone by decarboxylation. Acetoacetic acid is a normal intermediary in fat metabolism but it ordinarily exists only in a transitory state as acetoacetyl-coenzyme A in the anabolic and catabolic pathways of fatty acids (Fig. 1). Only the liver possesses an enzymatic organization which permits both the formation and the release of free acetoacetate into the blood stream.

The precursor of acetoacetyl-Co A is acetyl-Co A. This may be derived from a number of sources, but particularly from the catabolism of fatty acids. The latter occurs in series of steps illustrated on the right side of Figure 1. Each turn of the "fatty acid cycle" results in the release of a 2-carbon compound, acetyl-Co

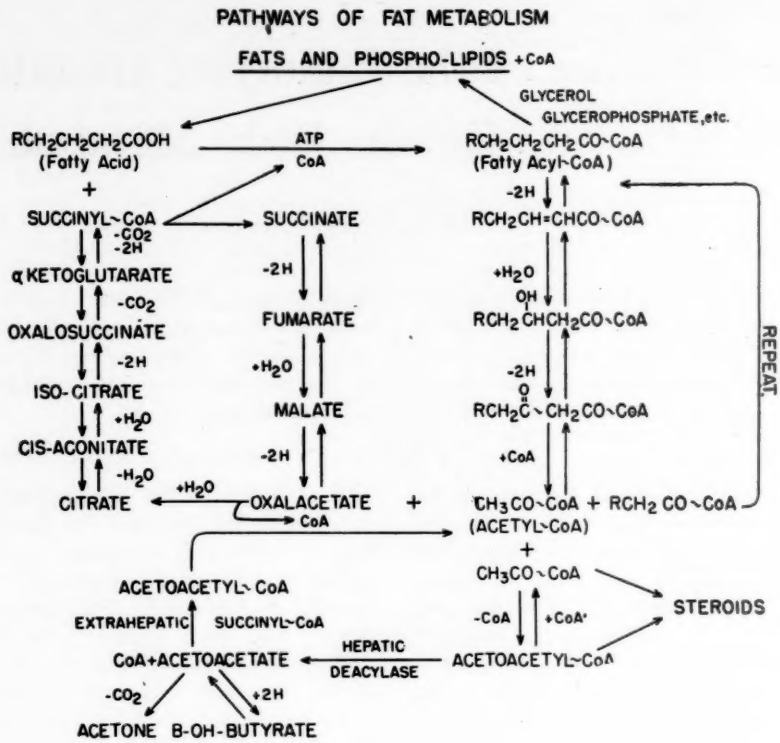


Figure 1.

A. If this is not further oxidized via the Krebs cycle, illustrated on the left side of the diagram, or resynthesized to fat, it may condense with another molecule of acetyl-Co A to form acetoacetyl-Co A, with the release of one molecule of coenzyme A. In the liver, the acetoacetyl-Co A is deacylated, through the influence of the enzyme deacylase, and released into the hepatic vein, freeing another molecule of coenzyme A. The extra hepatic tissues possess a very active enzyme system for recombining coenzyme A with acetoacetate which is brought to them via the blood stream or formed *in situ*. The coenzyme A may be donated by succinyl-Co A in the Krebs cycle. The liver lacks this enzymatic mechanism for reconstituting acetoacetyl-Co A and hence is the only organ which may present significant quantities of ketone bodies to the blood stream. The acetoacetyl-Co A formed in the extra hepatic tissues may be oxidized by way of the Krebs cycle or may be involved in synthetic pathways.

In the final analysis, ketone metabolism is an indirect reflection of the metabolism of acetyl-Co A, which in turn may be looked upon as the hub of intermediary metabolism (Fig. 2). This fact gives added interest to the study of ketone metabolism under various physiologic and pathologic circumstances. Acetyl-Co A may be derived from carbohydrate metabolism via pyruvate, from fat catabolism by successive $\beta$-oxidation of fatty acids and from protein through various possible pathways, as indicated in Figure 2. In turn the metabolism of acetyl-Co A may follow multiple alternate pathways. First, acetyl-Co A may be oxidized to completion in the Krebs cycle after condensation with oxalacetate to form citrate. With each turn of the cycle a molecule of acetyl-Co A is burned completely, oxalacetate is regenerated and a considerable amount of

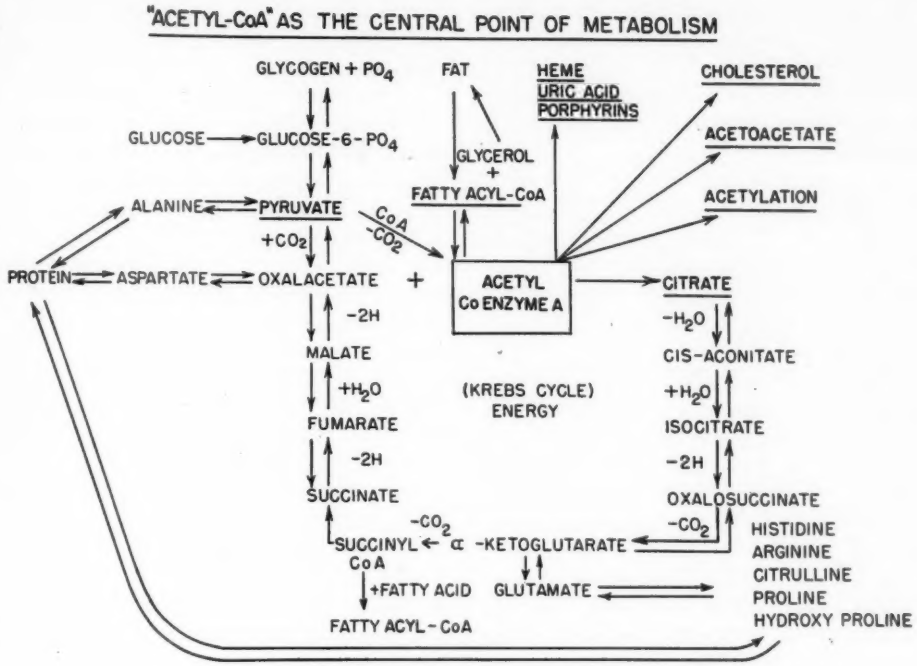


Figure 2.

energy is made available. Acetyl-Co A may also follow a synthetic path to longer chain fatty acids and eventually neutral fat, phospholipids, etc. (Fig. 1). This process depends on a continuing metabolism of glucose via the glycolytic pathway and the hexosemonophosphate shunt, the reduced diphosphopyridine nucleotide (DPNH) and reduced triphosphopyridine nucleotide (TPNH) generated thereby favoring fatty acid synthesis. Finally, acetyl-Co A is an intermediary in the synthesis of cholesterol and steroid hormones, purines, heme, and porphyrins, and is utilized in acetylation reactions.

Hepatic ketone body production may be interpreted in terms of a balance between the rates of production of acetyl-Co A from fat catabolism on the one hand and of disposal by oxidation by way of the Krebs cycle or by reduction and synthesis to fat on the other hand. Whenever its rate of formation exceeds that of its removal by oxidation or reduction, two molecules of acetyl-Co A may condense to form acetoacetyl-Co A and this, as well as additional acetoacetyl-Co A derived directly from fat catabolism, may be released into the hepatic vein as free acetoacetate through the action of the enzyme deacylase (Fig. 1). Since glucose utilization via glycolysis makes available DPNH for fat synthesis, it can be seen why utilizable carbohydrate exerts an antiketogenic action and why ketosis occurs during carbohydrate deprivation and in diabetes mellitus.

The level of blood ketone bodies is largely determined by their rate of production and release by the liver since their rate of utilization by the peripheral tissues apparently does not fluctuate within nearly as wide a range as does the rate of production. Renal function is another factor in ketosis, its major importance being that the ability to excrete ketone bodies may vary under different physiologic and pathologic circumstances. Ketonuria thus may not always be an adequate reflection of ketonemia.

With this background we may proceed to consider some aspects of the hormonal con-

trol of ketone metabolism. This has been a major research interest in this laboratory for several years and hence this report will be oriented primarily from the vantage point of our own studies, some of which are as yet unpublished.

### DIABETES MELLITUS AND INSULIN

The occurrence of ketosis and its alleviation by insulin in diabetes mellitus is so well known that it needs little discussion. The intensity of ketosis in diabetes far exceeds that of any other pathologic situation. It is the only type of ketosis in which a really serious disturbance in acid-base balance occurs and in which the accumulation of ketone bodies may result in notable symptoms, i.e., coma. In terms of current concepts of the metabolic lesion in diabetes mellitus, the basis for the ketosis seems reasonably clear.

In the absence of insulin, glucose utilization is inhibited either in its transfer through the cell membrane or in its phosphorylation to glucose-6-phosphate. Lipogenesis from carbohydrate is reduced sharply in the diabetic animal, presumably by a specific block in the conversion of acetyl-Co A to fat,[32] and the final oxidation of glucose by way of the Krebs cycle is depressed. Fat is mobilized to the liver and is catabolized at an accelerated rate. Stadie[33] has recently reviewed the evidence suggesting that the catabolic pathway and that the block in fatty-acid synthesis in diabetes may be between acetyl-Co A and butyryl-Co A. The combination of impaired glucose utilization and glycolysis, inhibition of fat synthesis and accentuation of fat catabolism makes for the maximal possible potential accumulation of acetyl-Co A in the liver. By the mechanisms already described the excess acetyl-Co A drains from the liver as acetoacetate. Insulin corrects the basic defect in carbohydrate utilization and fat synthesis and thus overcomes the ketosis, provided adequate carbohydrate is available for metabolism.

In the normal organism the direct metabolic influence of insulin in ketone metabolism must be distinguished from the secondary reaction to hypoglycemia. The immediate effect of insulin is to lower the level of blood ketones as the blood sugar level is falling; one may assume that this effect is brought about through a reversal of the biochemical mechanisms just described. However, once hypoglycemia is established the decline in blood ketone levels comes to a halt and is followed in several hours by an increase beyond the initial values, a sequence of events which has been known for many years.[34] In the normal subject the time of the ketosis corresponds to the period of recovery from hypoglycemia. This post-hypoglycemic ketonemia is due to an increased hepatic production of ketone bodies. Employing the technic of hepatic vein catheterization, we have made a quantitative estimate of the rate of splanchnic production of ketone bodies in man by simultaneously determining the hepatic vein-femoral arterial blood ketone body level difference and the estimated hepatic blood flow by the bromsulfthalein method.[35] Five hours after a brief episode of hypoglycemia induced in normal man by the intravenous injection of 0.1 unit insulin/kg body weight, there is a five-fold increase in ketone body production by the liver. (Table I).[36] This observation lends experimental support to the clinical experience that insulin hypoglycemia may initiate ketosis in the diabetic patient. In the same experiment an infusion of sodium octanoate, an 8-carbon fatty acid and known ketone body precursor, was made during the period of recovery from hypoglycemia to determine whether hypoglycemia resulted in an accelerated conversion of a fatty acid to ketone bodies. As is apparent from Table I, there was no greater increment in ketone body production over the basal values in hypoglycemic subjects compared to their controls, suggesting that the action of insulin hypoglycemia is not directly on fatty acid catabolism. This result contrasts with the action of a pituitary ketogenic factor to be described later which did result in a greater yield of ketone bodies from infused octanoate in the rat.[37]

A metabolic basis for hypoglycemia ketosis may be visualized readily in terms of current concepts of intermediary metabolism. Hypoglycemia, whether induced by insulin, phlorid-

zin or starvation, calls forth a metabolic adjustment which may be viewed teleologically as designed to restore the blood sugar as rapidly and effectively as possible to maintain the metabolic integrity of the central nervous system. This reaction to insulin hypoglycemia is essentially the same as that during fasting except that the former is of necessity more intense and is telescoped in time. The first response is a release of glucose from the preformed stores of glycogen in the liver.

TABLE I

Net Splanchnic Ketone Body Production in Man After an Overnight Fast and Five Hours After 0.1 Unit of Insulin per kg Body Weight Intravenously

| Subject group | Net splanchnic ketone body production μM acetone/min/M² | | |
|---|---|---|---|
| | Control | During Na. Oct. | Δ |
| Control | 34.0 ± 5.20 | 158.0 ± 6.86 | 114.0 ± 10.34 |
| Hypoglycemic | 160.7 ± 46.0 | 266.3 ± 43.5 | 105.6 ± 27.8 |
| p Values | <0.01 | <0.01 | >0.5 |

Measurements were made before and during an intravenous infusion of sodium octanoate. Note the marked increase in net splanchnic ketone body production in the individuals subjected to hypoglycemia. The increase in net splanchnic ketone body production during octanoate infusion is shown in the last column. This value was not influenced by the hypoglycemia.

Epinephrine facilitates this reaction, which is relatively short lived since the liver glycogen stores are rapidly depleted. Secondly, glucose utilization, particularly for fat synthesis, is reduced to a minimum in all tissues other than the central nervous system. An impaired tolerance to glucose is readily demonstrated following insulin hypoglycemia. Protein catabolism is accelerated, presumably for the purpose of gluconeogenesis. In the rat an increase in nitrogen metabolism has been shown to occur within three hours of the induction of hypoglycemia.[38] And finally fat catabolism is increased and with it ketosis occurs; the former is reflected in a lowering of the R.Q. This combination of metabolic events may be interpreted as "diabetes in miniature" and indeed in fasting has long been known as hunger or starvation diabetes. The ketosis is much less intense than in diabetic ketosis but presumably is brought about by somewhat similar mechanisms: i.e., the combination of diminished glucose utilization, impaired fat synthesis, and accelerated fat catabolism all leading to a surplus of acetyl-Co A to condense to acetoacetyl-Co A which in turn is released into the hepatic vein as acetoacetate. In contrast to diabetic ketosis, however, it is promptly alleviated by glucose administration.

There are reasons to believe that certain features of this metabolic reaction might be moderated by endocrine secretions since the total picture of starvation diabetes does not develop in the absence of the adenohypophysis or the adrenal cortex or both, and the response may be exaggerated in the absence of the pancreas. Moreover, an increased secretion of metabolic factors from the pituitary,[39] of adrenal cortical steroids,[40] and of epinephrine[41] has been reported during insulin hypoglycemia and it is well known that insulin secretion by the pancreas is depressed by hypoglycemia.[42] The concept that the endocrines are specifically involved in the ketosis of fasting and insulin hypoglycemia will now be examined.

### *The Adrenal Medulla*

Since an epinephrine discharge occurs early in response to hypoglycemia, it may be involved in the ketonemic response, particularly since the adrenal medulla has recently been implicated in certain types of fat mobilization to the liver.[43] The adrenal medulla, the chief source of epinephrine, is not essential for ketosis since the adrenodemedullated rat still exhibits a ketonemic response to insulin hypoglycemia.[44] Epinephrine itself, has a depressing effect on ketonemia in the rat,[45] although in other species no effect or a ketonemic response have been described.[46]

### *The Adrenal Cortex*

Early reports on the influence of adrenalectomy on ketosis were contradictory, largely because ketonuria was used as the basis for estimation of ketosis and the influence of the abnormal renal function of adrenal insuffi-

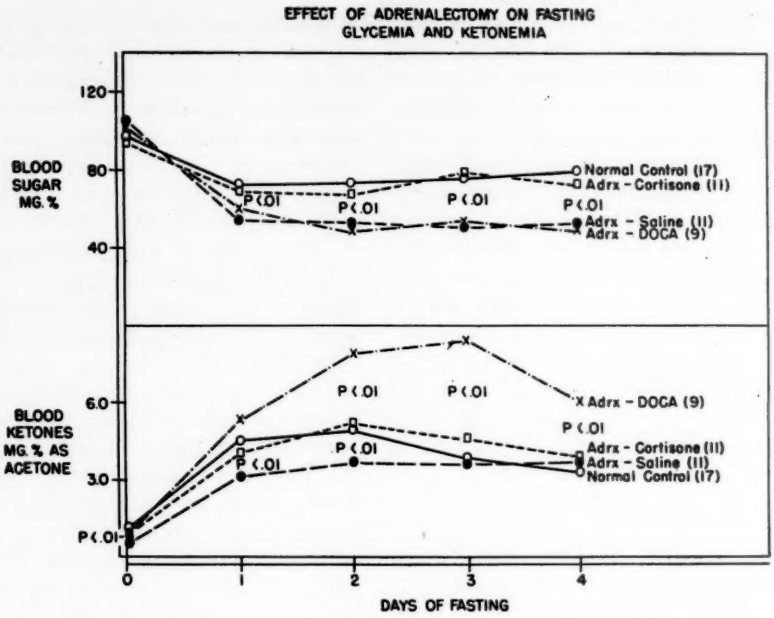


Fig. 3. Blood sugar and ketone body levels in normal and adrenalectomized rats during a four-day fast. Note the increased ketonemia in the DOCA-maintained rats and the decreased ketonemia in the saline-treated rats despite comparable hypoglycemia. Cortisone restores glycemia and ketonemia to normal.

ciency was not taken into account. Adrenalectomy reputedly depressed ketonuria but when blood ketone levels were measured, it was established that these were not as low as anticipated from the urine values.[20,47] Our studies have indicated that a failing peripheral circulation inhibits ketonemia and suggest that this factor may have been responsible for the difficulty in estimating the degree of ketonemia due specifically to the lack of adrenal steroids.[48] Indeed if adrenalectomized rats are kept in optimal condition with sodium chloride and desoxycorticosterone acetate (DOCA) during a prolonged fast, they exhibit a slightly but significantly greater ketonemia than do normal rats[49] (Fig. 3). This ketosis is reduced to normal by cortisone treatment. DOCA itself has no effect on ketonemia in normal rats and hence, presumably is not responsible for the ketosis observed in the DOCA maintained adrenalectomized rats. Not only is there enhanced ketonemia in the fasted adrenalectomized rat protected with DOCA and salt, but recent data also indicate that the same type of rat can still respond to insulin hypoglycemia with a rise in blood ketones, despite the depressing effect of hypoglycemia on the circulation of the adrenalectomized rat.[44]

It is thus apparent that the adrenal cortex, like the medulla, also is not essential for the ketosis of fasting or hypoglycemia, even though their secretion is increased by these stimuli. Indeed, as first shown by Kinsell[50] in man with cortisone and a slow infusion of corticotropin (ACTH) and confirmed by us[49,51] in the rat, cortisone and related compounds actually inhibit fasting ketosis (Fig. 4). Cortisone and hydrocortisone also suppress the ketosis associated with cold stress[51] and intoxication with sodium fluoroacetate, a Krebs cycle poison,[52] but they do not modify the ketonemia secondary to insulin hypoglycemia in either man[36] or the rat,[44] or that due to certain pituitary extracts in the rat.[45] It is thus apparent that the adrenal cortex not only is

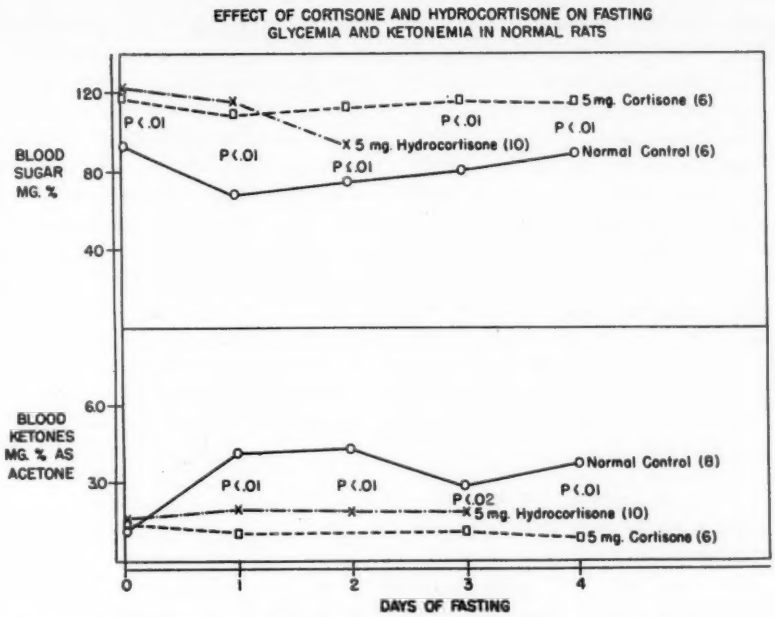


Fig. 4. Suppression of fasting ketosis by cortisone and hydrocortisone. Note elevated blood sugar levels in corticoid treated rats.

not essential for ketosis, but the glucocorticoids may, under certain circumstances, actually inhibit ketosis.

The possible mechanisms by which the adrenal cortex influences ketone metabolism are difficult to formulate, largely because there are still many gaps and certain contradictions in our knowledge of the role of the adrenal cortex in intermediary metabolism. The largest area of disagreement relates to the role of the adrenal cortex in fat anabolism and catabolism. Some of the discrepancies undoubtedly hinge on differences in experimental conditions and technics. Thus the data of Welt and Wilhelmi[53] indicate that adrenalectomized rats fed a fat-free high carbohydrate diet have a higher rate of lipogenesis from carbohydrate than controls, but that their rate of fat utilization is also considerable. These changes were the reverse of those following ACTH treatment in normal rats. This, as well as the observation that adrenalectomy and hypophysectomy restore lipogenesis to normal in the diabetic animal,[54] indicate that adrenal hormone lack has a favorable influence on lipogenesis. This interpretation, however, is not supported by the studies of Lipsett and Moore[55] and Perry and Bowen[56,57] which present evidences of impaired lipogenesis from acetate and no disturbance in fatty acid catabolism in the livers of adrenalectomized rats.

In either case, one can offer a reasonable explanation for the mild ketosis of fasting in the adrenalectomized animal and the suppression of ketosis by glucocorticoids. In the former, the stage is set by the rapid depletion of liver glycogen during fasting, due presumably to continuing ample glucose utilization without compensatory gluconeogenesis from protein catabolism as occurs normally during a fast. As the fast continues lipogenesis must decline and fat catabolism is at least as great, or greater than normal. Both these factors contribute to ketogenesis. If in addition, we assume that the accelerated protein catabolism which occurs in the normal organ-

ism can inhibit ketosis to some degree by contributing intermediaries to the Krebs cycle, it becomes apparent why ketonemia in the adrenalectomized rat finally exceeds the normal as the fast continues.

The antiketogenic effect of the glucocorticoids may be interpreted in terms of their action in shifting the balance of fat metabolism toward a net accumulation of fat, regardless of whether this is brought about by an inhibition of fat synthesis and fat catabolism with the latter inhibition exceeding the former or whether fat synthesis may actually be stimulated. In any case, the consequence of such a shift would be a decreased supply of acetyl-Co A and acetoacetyl-Co A from fat catabolism. At the same time the accelerated rate of gluconeogenesis associated with glucocorticoid action during fasting maintains liver glycogen and also keeps up a supply of Krebs cycle intermediaries from amino acids to help "drain off" acetyl-Co A into the cycle despite a simultaneous mild inhibition of glucose utilization. Cortisone has diabetogenic activity, but the bulk of evidence indicates that it causes a type of diabetes which is primarily due to overproduction of carbohydrate,[58] in contrast to the predominantly "under-utilization" diabetes of insulin deficiency.[59] It is of interest that the diabetes of Cushing's disease is rarely complicated by ketosis. Moreover, ACTH or cortisone has been given to diabetic patients and has exacerbated glycosuria without inducing ketosis.[60] Indeed in some cases ketosis has even been diminished by cortisone treatment.

The demonstration by Kinsell[50] that a continuous infusion of ACTH inhibited fasting ketosis in man seemed at first to be a paradox since ACTH had been reported to be ketogenic in the rat.[61] Studies in this laboratory at first heightened the controversy because it was found that ACTH injected into rats four times daily had no effect whatsoever on ketonemia during a four-day fast even though cortisone in a comparable experiment completely suppressed ketosis.[51] Eventually, however, the contradictory data were explained by the discovery that in acute experiments ACTH produced an immediate rise in blood ketones and that this ketonemia occurred even in the *total* absence of the adrenal glands.[62] In short, the previously described ketogenic effect of ACTH was not mediated by corticoids from adrenal stimulation but was due either to an extra-adrenal metabolic effect of ACTH or to contamination of the ACTH with a ketogenic principle from the pituitary. Kinsell's results, in turn, were obviously due to the mode of administration of ACTH employed by him. The slow infusion resulted in a strong stimulation of the adrenal cortices with a minimal dose of the pituitary factor. These observations lead us to a consideration of pituitary factors in ketosis.

### ADENOHYPOPHYSIS

It is well known that certain pituitary extracts not only induce ketosis but also have other interesting metabolic effects. These include mobilization of fat to the liver (adipokinesis), hyperlipemia, depression of the R.Q., hypoglycemia in the fasted animal and anti-insulin activity.[63] As pituitary extracts became more purified these various metabolic activities became increasingly associated with the fraction possessing growth activity and, indeed, most investigators are of the opinion that they are specific properties of "growth hormone." On the other hand extensive chemical and biologic studies in at least two laboratories have yielded evidence to indicate that the metabolic activities noted above may be as much or more a property of a protein or peptide molecule that behaves chemically more like corticotropin than somatotropin.[64,65] These metabolic activities of corticotropin are clearly not mediated by adrenal cortical stimulation, being demonstrable in adrenalectomized animals. However, it is not established whether they represent properties of the same molecule or portion thereof which is responsible for the corticotropic activity per se. Preliminary data suggest that ketogenic and adipokinetic activities may persist after chemical treatment which abolishes adrenal ascorbic acid depleting activity. Furthermore, recent studies have shown that of four peptides derived by peptic digestion of Oxycel-corticotropin and possessing cortico-

tropic activity, only two have ketogenic activity.[45] The ketogenic activity does not seem to represent contamination with growth hormone *per se* since chemical treatment which destroys the growth-promoting and diabetogenic effects of growth hormone does not eliminate the ketogenic response to either ACTH or growth hormone.[45]

The problem of the nature of the pituitary factor(s) concerned with fat mobilization and ketosis is further confounded by the fact that thyroid-stimulating hormone also has these properties and has, indeed, been considered by some as the fat-mobilizing and ketogenic hormone.[66,67] It is apparent that while all investigators agree that the adenohypophysis influences fat metabolism, there is at present no agreement concerning the identity of the factor or factors involved. From studies in our laboratory we are led to the preliminary view that growth hormone, corticotropin and thyrotropin each independently have ketogenic activity. It remains to be established whether the ketogenic and other metabolic activities of different pituitary preparations represent true physiologic properties of hormones or whether some or all might be artefacts resulting from the chemical manipulation of pituitary proteins and peptides. They may depend on specific amino acid sequences which endow the whole or a fragment of the original hormone molecule with these biologic actions. Precedent for such a concept has just been published by Harris & Roos[68] and Geschwind, *et al*,[68a] who showed that melanocyte-stimulating hormone-(MSH) and ACTH have a certain amino acid sequence in common. They suggest that the latter might be responsible for intermedin-like activity of corticotropin preparations.

Because the adenohypophysis apparently secretes a substance or substances which stimulate ketosis and since the adrenal cortex and medulla are not necessary for the acute ketosis of hypoglycemia, it has seemed quite reasonable to postulate that the pituitary gland is the endocrine mediator in this response. This is logical since it had been considered well-established that the hypophysectomized animal does not readily develop ketosis. It came as a surprise, therefore, to find that the hypophysectomized rat developed ketonemia almost as easily as the normal animal during and following hypoglycemia (Fig. 5).[44] Moreover, when the proposition that ketosis does not develop during a fast in the absence of the hypophysis was re-

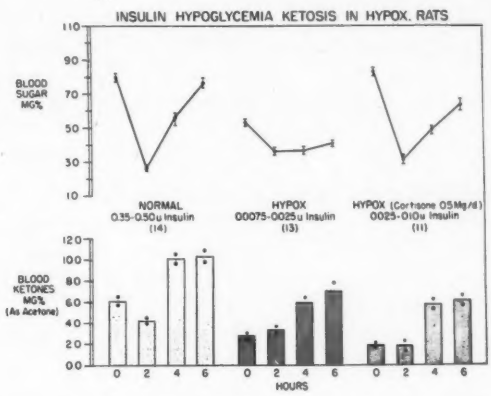


Fig. 5. Blood ketone levels during and following insulin hypoglycemia in normal and hypophysectomized rats. Although the absolute levels of ketone bodies are lower in the hypophysectomized rats, the increment from the starting level after hypoglycemia was not significantly different in the three groups. Note the fall in ketone bodies at two hours in the controls. This did not occur in the hypophysectomized rats.

examined, it was found that this is true only during a short fast; after 72 hours the hypophysectomized animal exhibits greater ketonemia than the intact animal (Fig. 6). In the normal rat the peak of ketonemia occurs by about the forty-eighth hour; in the hypophysectomized and in the adrenalectomized rat ketonemia tends to increase progressively during the fast. The ketosis in the hypophysectomized rats is associated with greater and more sustained hypoglycemia than in the normal animal. The fall in ketone levels in the normal fasting animal corresponds with the phase of rising blood sugar (and presumably of liver glycogen) and hence there are reasons to suspect that the adrenal cortex might be responsible for the metabolic adjustment, since these changes do not take place in the absence of the adrenal cortex. On the other hand, however, Tepperman and Tepperman[69] found that ketone body produc-

tion by liver slices from hypophysectomized rats was depressed. During the first 15 days after hypophysectomy ketogenesis could be restored by treatment of the rats with growth hormone. Beyond 30 days after hypophysectomy, cortisone and growth hormone were necessary to restore ketogenesis. Our experience contrasted with Tepperman's for hypophysectomized rats showed an even greater

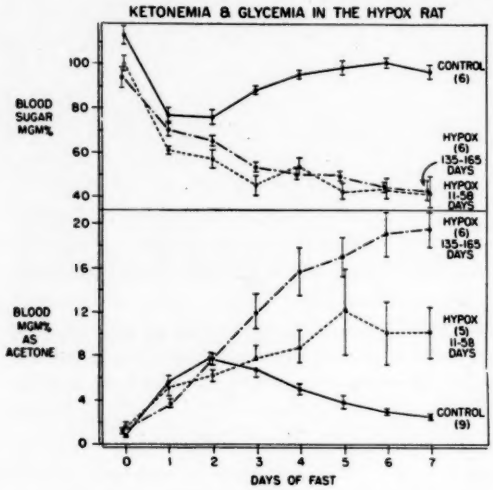


Fig. 6. Blood sugar and ketone values during a seven-day fast in normal and hypophysectomized rats. Note the inverse relationship between glycemia and ketonemia, and the greater ketonemia in the rats that had been hypophysectomized the longest.

ketonemic response 135 to 165 days after operation than occurred 11 to 58 days after operation. Rats recently operated upon exhibited more weight loss during fasting which we interpret as indicating the persistence of some adrenal function (Fig. 7). Later when adrenal atrophy was complete, ketosis was greater and weight loss less during the fast. Bondy and Wilhelmi[70] introduced another parameter into this problem when they found that thyroid treatment restored to normal the low ketogenic potentialities of the liver slice from the hypophysectomized rat. Our own data on the influence of thyroid treatment on ketosis in the hypophysectomized rat are indecisive.

These data appear to be at variance with earlier reports showing that hypophysectomy suppressed fasting and phloridzin-poisoning ketosis.[12] However, examination of the data indicates that the discrepancy is not as great as it seems. In no previous study was a prolonged fast carried out, the longest on record in which blood ketone levels were measured being only 36 hours.[71] In this study ketonemia was the same in the controls and the hypophysectomized animals at this time, even though the latter lagged behind the controls prior to this. In our study on the ketosis of the hypophysectomized rats, ketonemia did not clearly exceed that of the controls until after 72 hours. More difficult to reconcile with these results are the well established data of Houssay showing that hypophysectomy abolishes the ketosis of pancreatic diabetes.[12]

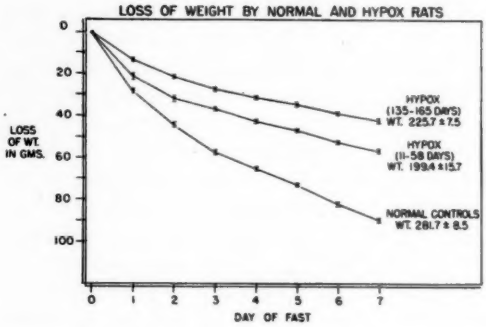
LOSS OF WEIGHT BY NORMAL AND HYPOX RATS
LOSS OF WT. IN GMS.
HYPOX (135-165 DAYS) WT. 225.7 ± 7.5
HYPOX (11-58 DAYS) WT. 199.4 ± 15.7
NORMAL CONTROLS WT. 281.7 ± 8.5
DAY OF FAST

Fig. 7. Weight loss of rats whose blood sugar and ketone data are shown in Figure 6. The weights at the beginning of the fast are shown on the right.

The mechanism(s) by which pituitary factors induce ketosis have not been elucidated. There are a number of possibilities which have been considered in detail elsewhere;[64] the problem is complicated by the question of whether or not there are several different ketogenic pituitary factors. However, they all seem to have one property in common, i.e., they also cause a mobilization of fat from the periphery to the liver, thereby providing an increased substrate for ketone production in that organ. In the case of growth hormone the fat accumulation in the liver is preceded by an increase in the blood neutral fat content.[72] The same agent lowers the R.Q.,

inhibits glucose utilization for fat synthesis and oxidation, stimulates fat catabolism, blocks insulin action and promotes protein anabolism, all of which would be expected to throw the balance in favor of ketone body production. Indeed, with respect to the last and specific action of growth hormone, it might be noted that under most circumstances of rapid protein synthesis, as in childhood, pregnancy, lactation and the anabolic phase of convalescence, ketosis develops more readily than normally during fasting. This may be a reflection of the fact that under these circumstances the metabolic machine is geared to a rapid breakdown of fat and to a slowed rate of protein breakdown and gluconeogenesis.

The ketogenic factor which is chemically associated with corticotropin but does not act through the adrenal cortex likewise promotes fat mobilization and also may have a direct effect on fatty acid catabolism and on ketone body production *per se*.[37] This was shown in a study in which pre-treatment with a single dose of corticotropin resulted in a significantly greater increase in blood ketones during an infusion of sodium octanoate in the rat than would be expected from either the octanoate itself or the hormone. Comparable studies have not yet been done with purified growth hormone to determine whether it has a similar action on fatty acid metabolism. Corticotropin also prevented the antiketogenic effect of a glucose load. Like growth hormone, corticotropin may also lower the blood sugar during fasting but this reaction is not responsible for the ketosis since the latter precedes the hypoglycemia.[62] It also improves carbohydrate tolerance during an intravenous glucose tolerance test[37] possibly by promoting glycogen storage in muscle since there are evidences that it may have an anti-insulin effect in other regards.[64]

The mechanism by which thyroid-stimulating hormone promotes fat mobilization and ketosis is even less well understood. The thyroid is not necessary for this response. Lotspeich and Petersen[66,67] consider the lowering of the liver glycogen and blood sugar by this material as well as growth hormone to be important for ketosis.

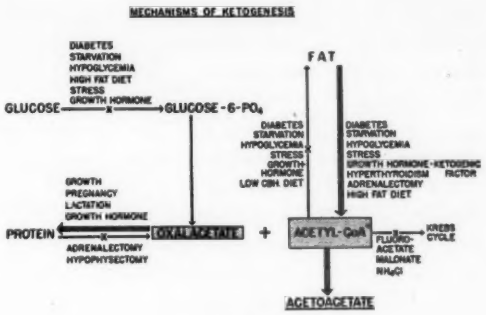


Fig. 8.

The observation that the hypophysectomized rat eventually exhibits a brisk ketosis during fasting or after insulin hypoglycemia requires some revision of our notions concerning the role of the adenohypophysis in ketone metabolism as well as of our concepts concerning the specificity of the ketogenic response. At first it would seem paradoxical that both excess and deficiency of pituitary hormones should be associated with ketosis. However, more sober thought brings us to the realization that this finding is entirely consistent with a basic principle of endocrinology, namely that the hormones do not initiate or stop metabolic reactions but simply modify their rates. The fasting hypophysectomized animal becomes profoundly hypoglycemic, depleting its muscle and liver glycogen stores and at the same time has difficulty in mobilizing protein rapidly enough for gluconeogenesis because of adrenal insufficiency. Under these circumstances this carbohydrate deprived animal has little choice but to draw on its fat stores, which may be ample until fasting,

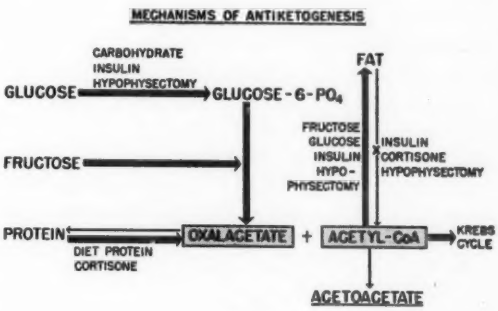


Fig. 9.

but even these cannot be oxidized completely in the liver because of carbohydrate lack; ketosis is the consequence. It is important to emphasize that the ketosis still develops more slowly than normal and that in terms of the mangitude of the hypoglycemic stimulus, the degree of ketosis may likewise still be less than normal; a conclusion entirely consistent with the basic principle of endocrinology just stated. Indeed, a more appropriate control for the hypophysectomized rat might be the intact phloridzinized rat with comparable hypoglycemia. It might be predicted from data already available that the normal animal during a prolonged fast would show a marked increase in ketosis during phloridzin hypoglycemia while the hypophysectomized rat would show little or no change beyond that already noted during the fast.

Some of the endocrine and nutritional factors which influence ketogenesis and antiketogenesis in the liver are depicted in Figures 8 and 9. These diagrams reduce the phenomenon of ketosis to its simplest terms, i.e., the balance between production and utilization of acetyl-Co A by diverse pathways. Ketogenesis is represented by the situation in which there is a theoretic excess of acetyl-Co A which "drains off" as acetoacetate. Conversely antiketogenesis follows when the metabolic pattern favors utilization of acetyl-Co A by fat synthesis and oxidation in the Krebs cycle. As indicated in the diagrams, nutritional and hormonal factors may influence different steps in this metabolic picture, directly or indirectly and in diverse ways.

## SUMMARY

Current concepts of intermediary fat metabolism and ketogenesis have been reviewed. The mechanisms involved in the ketosis of diabetes mellitus, fasting and insulin hypoglycemia and the antiketogenic action of glucose and insulin are discussed. In addition, the possible roles of the endocrine glands in fasting and insulin hypoglycemia ketosis are reviewed.

Neither the adrenal medulla, the adrenal cortex or hypophysis are essential to the development of ketosis during fasting or hypoglycemia. Glucocorticoids suppress the ketosis resulting from fasting, cold-stress and fluoroacetate poisoning but not that due to insulin hypoglycemia or ketogenic pituitary extracts.

Growth hormone, thyrotropin and corticotropin possess ketogenic and adipokinetic action, the latter independent of the adrenal cortices. The significance of multiple ketogenic factors and their possible modes of action are considered. The hypophysectomized rat develops greater ketosis and more hypoglycemia than does the normal rat during a seven-day fast.

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# The Relationship of Vitamin $B_{12}$ to Carbohydrate Metabolism and Diabetes Mellitus

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THE PHYSIOLOGIC function of vitamin $B_{12}$ is as yet far from being fully understood. Experimental difficulties arising from the lack of a procedure to insure the development of a pure vitamin $B_{12}$ deficiency in animals and from the multiplicity of physiologic functions of vitamin $B_{12}$ tend to impede progress. Mere feeding of test animals on a vitamin $B_{12}$-free diet does not necessarily result in a development of vitamin $B_{12}$ deficiency, because of the common practice of coprophagy which must be strictly prevented. Even when fed on the same diet, the age or the previous history or the vitamin $B_{12}$ reserve of the test animals may produce diametrically opposite clinical symptoms. For example, feeding of a soybean meal diet retards the growth of weanling rats but produces obesity in adult rats which tend to retain water and deposit fat. Although the importance of vitamin $B_{12}$ as a hematopoetic or growth-stimulating agent is well recognized, it does not necessarily follow that vitamin $B_{12}$ deficiency in man must always be accompanied by hematopoetic and neurologic symptoms of pernicious anemia.

That vitamin $B_{12}$ plays a dominant role in the utilization of carbohydrates is now well established. The present communication will summarize (1) this aspect of the metabolic role of the vitamin, (2) the interrelationship between vitamin $B_{12}$ absorption and endocrine function, and (3) the relationship of vitamin $B_{12}$ to diabetes mellitus.

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## THE METABOLIC ROLE OF VITAMIN $B_{12}$

By way of review, it may be mentioned that the hypothesis that vitamin $B_{12}$ is important for carbohydrate or fat metabolism is supported by the following experimental facts (Table I).

TABLE I

Vitamin $B_{12}$ and Carbohydrate Utilization

| | Vitamin $B_{12}$ | |
|---|---|---|
| | Treated | Untreated |
| Increase in body wt. (g) of weanling rats on high-carbohydrate, low-fat diet | $87 \pm 4.9$<br>$155 \pm 5.7$ | $56 \pm 9.8$ (28 days)<br>$108 \pm 6.5$ (63 days) |
| Fat content of carcass (%) | $16.6 \pm 0.71$ | $4.3 \pm 0.58$ |
| Vitamin $B_{12}$ serum levels ($\mu\mu$g/ml) | | |
| glucose injected | $0.224 \pm 0.084$ | |
| saline injected | $0.662 \pm 0.120$ | |
| Blood glucose mg/100 ml | $110 \pm 10$ | $380 \pm 21$ |

(1) Weanling rats[1] require more vitamin $B_{12}$ for growth if their diet is rich in carbohydrate and low in fat.

(2) The analyses of the chemical composition of the carcass of vitamin $B_{12}$-deficient rats[2] reveal an abnormally low percentage of fat which can be elevated by vitamin $B_{12}$ administration.

(3) Glucose administered by the parenteral route[3] to rats placed on high-carbohydrate and

low-fat diets results in a decrease in the vitamin $B_{12}$ level in plasma and in liver.

(4) Deficiency of this vitamin in rats[4] is usually accompanied by hyperglycemia which can be correlated upon the administration of vitamin $B_{12}$.

These experiments suggest that, vitamin $B_{12}$ plays an important role in the metabolism of carbohydrates *in vivo*. Therefore, it is of interest to examine *in vitro* whether vitamin $B_{12}$ deficiency will cause any impairment of enzyme systems necessary for carbohydrate metabolism. It was found that the erythrocytes of vitamin $B_{12}$-deficient animals contain less glutathione[5] or enzyme[6] activities essential for the degradation of glucose to ribose. To demonstrate this latter phenomenon, the erythrocytes of vitamin $B_{12}$-deficient rats were suspended in a buffered solution of glucose and the ribose formation or oxygen uptake was measured.[4] The rate of glucose degradation by the red blood cells of deficient animals was much less than that of treated ones (Table II). The rate was increased upon the addition of glutathione to the enzyme system but not of vitamin $B_{12}$. These data taken as a whole demonstrate that this particular vitamin deficiency brings about a decrease in the glutathione content of the red cells, and thus inactivates the enzyme system important in the degradation of glucose to ribose.

TABLE II

Effect of Glutathione on Ribose Formation

| | Vitamin $B_{12}$-deficient ($B_{12}$-injected) | Vitamin $B_{12}$-deficient | Vitamin $B_{12}$-deficient glutathione |
|---|---|---|---|
| Ribose before incubation | 280* | 277* | 274* |
| Ribose after incubation | 550 | 466 | 600 |
| Increase in ribose | 270 | 189 | 326 |
| $Qo_2$/hr/ml packed cells | 32 | 14 | 35 |

*Ribose expressed μg/ml packed erythrocytes.

The effects of this deficiency on other enzyme systems important to carbohydrate metabolism have also been investigated. It was found that vitamin $B_{12}$ deficiency in chicks caused an elevation of the coenzyme A content in the liver.[7] Our recent findings[8] suggest that vitamin $B_{12}$ deficiency will cause a marked decrease in the reduced form of DPN with the concomitant increase in the oxidized form in the liver of rats. Thus, the ratio of the oxidized to the reduced forms becomes highly elevated. Since glutathione, CoA and DPN systems are involved in many metabolic pathways, available data do not, as yet, provide us with a sufficient clue to speculate about the pathway in carbohydrate metabolism impaired by vitamin $B_{12}$ deficiency. They do, however, demonstrate the importance of this nutrient in the maintenance of balanced enzyme systems essential for the utilization of carbohydrate and the transformation of fat.

## HORMONAL INFLUENCE OF VITAMIN $B_{12}$ ABSORPTION AND UTILIZATION

The utilization or action of vitamins depends to a very large extent on the endocrine activities of the test animals. Some interrelationships between vitamin $B_{12}$ absorption and endocrine activity will now be discussed.

### *Effect of ACTH or Cortisone*

Prolonged injection of cortisone or corticotropin (ACTH) into normal rats will bring about hyperglycemia correctable by the injection of vitamin $B_{12}$.[9] If the hormone and the vitamin are given simultaneously, no hyperglycemia will result. Pre-administration of cortisone to rats also decreases the retention of injected radioactive vitamin $B_{12}$. In a typical experiment, two groups of mature female rats (average 220 g) raised in our own colony, were employed. Group A received daily except Sunday 5 mg of cortisone suspension per rat per day for three weeks. Group B, serving as control, received on the same days an injection of one ml of isotonic saline solution. After this pretreatment for three weeks, each animal in both groups was given a test dose of one μg of radioactive vitamin $B_{12}$. The urinary specimens collected 24 hours before as well as 24 and 48 hours after injection were assayed for radioactivity and for microbiologic activity of vitamin $B_{12}$. Forty-eight hours after the injection of the vitamin the animals were sacrificed and tissues such as liver, kidneys and thigh muscle were removed for radioac-

TABLE III

Radioactivity in Urine and Tissues

| Group | Treatment | Vitamin $B_{12}$ in urine | | mγ Vitamin $B_{12}$-bound/g tissue | | |
|---|---|---|---|---|---|---|
| | | R | M | Muscle | Liver | Kidneys |
| A | Cortisone | 0.72 | 0.77 ± 0.03 | 0.11 | 5.7 | 167 |
| B | Saline | 0.44 | 0.48 ± 0.03 | 0.18 | 11.0 | 244 |

R = Micrograms of vitamin $B_{12}$ as measured by the radioactivity in the 24-hour urine specimen.
M = Micrograms of vitamin $B_{12}$ as measured by the microbial activity in the 24-hour urine specimen.

tivity determinations. The results of this experiment (Table III) demonstrate that the vitamin $B_{12}$ activity in the 24-hour urine specimens of the cortisone treated animals was twice that of the control animals, as measured either by the microbiologic activity or by the radioactivity. The results of tissue analyses demonstrate that in each instance the organs of the treated animals were less radioactive than those of the saline-injected controls.

Another effect of cortisone administration is elevation of the vitamin $B_{12}$ serum level.[10] In a typical study, 20 adult rats were divided into two groups. Group A (ten rats) received 5 mg of cortisone daily for three weeks and group B (ten rats) received saline solution likewise by injection. Shortly before and one week after the last injection of cortisone or saline, all animals were bled and plasma specimens were analyzed for vitamin $B_{12}$ activity. The results (Table IV) showed a marked elevation of vitamin $B_{12}$ level in the hormone-treated group. Similar effects were observed following the administration of carbon tetrachloride and these results led us to believe that cortisone or carbon tetrachloride causes injury to liver with subsequent release of vitamin $B_{12}$. This belief was substantiated by the fact that the liver vitamin $B_{12}$-content of treated animals is about one-half that of the control rats.

Another effect of cortisone administration is the lowering of glutathione in the erythrocytes. In one experiment, 20 male adult rats were divided into two groups. Group A received daily, except Sunday, five mg of cortisone suspension per rat per day for five weeks. Group B received on the same days injections of an isotonic saline solution. The animals were then bled and the glutathione contents in the red blood cells were determined according to the procedure described previously.[5] The mean values of glutathione in the cells were 288 ± 40 and 166 ± 32 μμ moles/100 ml of cells for the control and cortisone-treated animals, respectively.

TABLE IV

Plasma Vitamin $B_{12}$ Levels of Rats

| Dosage | Plasma vitamin $B_{12}$ level (mγ/ml) | |
|---|---|---|
| | After 24 hours | After 7 days |
| $CCl_4$ in ml/100 g body wt (intravenous) | | |
| 0 | 0.66 ± 0.024 | 0.775 ± 0.025 |
| 0.035 ml | 0.87 ± 0.082 | 0.809 ± 0.031 |
| 0.070 ml | 1.17 ± 0.105 | 0.857 ± 0.025 |
| Cortisone (subcutaneous) | | |
| 0.0 | 0.65 ± 0.018 | 0.702 ± 0.032 |
| 1.0 mg | 1.27 ± 0.011 | 1.00 ± 0.102 |

### *Effect of Thyroid*

Thyroidectomy in rats prevented the absorption of orally administered radioactive vitamin $B_{12}$. The absorption could be restored in such animals by incorporating desiccated thyroid in the diet. This substance, if withdrawn, would again result in impairment of absorption of vitamin $B_{12}$. This phenomenon was studied in rats in different states of thyroid activity.[11] Fifty millimicrograms of $Co^{60}$-labeled vitamin $B_{12}$ were given to test animals by stomach tube and feces were collected for six days for the determination of radioactivity. The difference between the administered and excreted radioactivity was taken as a measure

of the absorbed vitamin $B_{12}$. The data show that thyroidectomy resulted in a decrease of absorption from 25.5 ± 1.0 mμg (before thyroidectomy) to 6.2 ± 1.1 mμg (after thyroidectomy). When the thyroidectomized rats were subsequently given a stock diet containing 0.1 per cent desiccated thyroid for three weeks, the absorption was increased to normal levels (27.7 ± 1.3 mμg). This increase in absorption was also reflected in the greater content of radioactivity in various organs. The radioactivity in the livers and kidneys was 7.0 ± 0.2 mμg and 4.7 ± 0.3 mμg for the thyroid-treated rats and 3.4 ± 0.4 mμg and 3.2 ± 0.4 mμg for the thyroidectomized rats, respectively. It is obvious then, that thyroid activity plays an important role for the absorption of this vitamin.

The results presented above demonstrate the complexity in the metabolic processes and interdependence of vitamins and hormones. This conclusion is likewise borne out from the results of the studies of Meites[9] and his associates. They found likewise that the administration of cortisone acetate to young male rats brought about a loss in body weight, reduction in hair growth and a decrease in thymus weight. When the intake of vitamin $B_{12}$ was increased several fold above the amounts usually required by young rats for normal growth, the ill effects of large doses of cortisone was partially or largely counteracted. They likewise found that vitamin $B_{12}$ could correct the pronounced hyperglycemia and glycosuria which they called cortisone diabetes.

## VITAMIN $B_{12}$ AND DIABETES MELLITUS

What is the relationship between vitamin $B_{12}$ and diabetes? The implication of pituitary hormones and in particular the hypersecretion of the adrenals was championed by the late Dr. Friedenwald and his associates.[12,13] Their hypothesis is now finding wider experimental support, and will be discussed later.

We would like to summarize briefly some of our own studies on the handling of vitamin $B_{12}$ by diabetics with and without retinopathy and cite several important studies undertaken by other investigators working in this field at the Johns Hopkins Hospital.

### *Retention of Vitamin $B_{12}$*

This study was conducted in collaboration with Dr. Bernard Becker,[14] and all the patients were drawn from the Wilmer Ophthalmological Institute and the Diabetic Clinic of the Johns Hopkins Hospital. When the diagnosis of diabetes had been established and the presence or absence of retinopathy recorded, all patients received a single dose of 50 μg or 65 μg vitamin $B_{12}$ by the intramuscular route. Insulin therapy was not interrupted during these studies. All estimations of vitamin $B_{12}$ in urine were made microbiologically, and radioactively in some cases where radioactive $B_{12}$ was given. The results of our original study as well as of a subsequent one are given in Table V. In this table, the microbial activity of vitamin $B_{12}$, expressed in micrograms, in the urine specimens of diabetics with and without retinopathy are tabulated. It is clearly demonstrated that in both studies I and II, there was a uniformly greater excretion by subjects with retinopathy than by those without retinal lesion. The urinary excretion of normal subjects was included for comparison. The data show that in both series the diabetics with retinopathy excreted an average of approximately 20 μg of vitamin $B_{12}$, and subjects without retinopathy excreted only 4 μg; whereas, the normal subjects excreted 10 μg. Statistical analyses indicate a significant difference, not only between the two groups with this disease, but also between either

TABLE V

Urinary Excretion after Injection of Vitamin $B_{12}$ (50 μg)*

| Study | Retinopathy | | Non-diabetic controls |
|---|---|---|---|
| | Absent | Present | |
| I‡ | 4.2 ± 1.7 (13)† | 19.0 ± 2.1 (22) | 9.6 ± 1.4 (6) |
| II | 3.9 ± 2.2 (5) | 20.4 ± 3.6 (4) | 10.4 ± 1.8 (10) |

* Expressed as micrograms of vitamin $B_{12}$ excreted in 24-hour urine specimens.

† Numbers in parenthesis indicate the number of subjects studied.

‡ See reference 14. Data in study II have not been published previously.

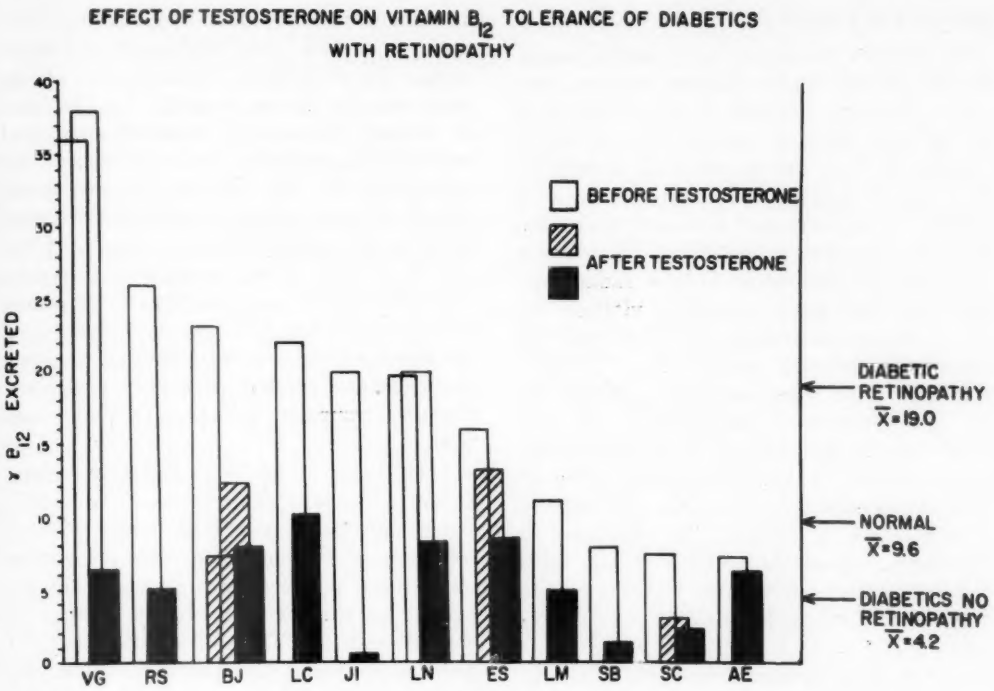
EFFECT OF TESTOSTERONE ON VITAMIN B12 TOLERANCE OF DIABETICS WITH RETINOPATHY
BEFORE TESTOSTERONE
AFTER TESTOSTERONE
% B12 EXCRETED
DIABETIC RETINOPATHY X̄=19.0
NORMAL X̄=9.6
DIABETICS NO RETINOPATHY X̄=4.2
VG RS BJ LC JI LN ES LM SB SC AE

Fig. 1.

one of them and the non-diabetic subjects.

Testosterone has been used in the management of diabetics with retinopathy.[15] It was, therefore, of interest to study the effect of this hormone on the excretion of vitamin $B_{12}$. To this end, a group of diabetics with retinopathy were first given one or occasionally two tests with vitamin $B_{12}$ (50 $\mu$g) to establish excretion levels before androgen therapy. After intramuscular injections of 100 to 200 mg testosterone cyclopentylpropionate (Depo-Testosterone®) every two to three weeks, these subjects were again given one or sometimes more tests for vitamin $B_{12}$ excretion. Successive tests, when made, were at intervals of at least four weeks. Only after the completion of the microbiologic assays for vitamin $B_{12}$ activity in urine was the schedule of testosterone administration compared. The results of such a study are presented in Figure 1. Among the 11 subjects studied there was in every instance a significant decrease in vitamin $B_{12}$ excretion following the testosterone administration, although the magnitude of this decrease varied from one individual to another.

These results may be summarized as follows: after an intramuscular test dose of vitamin $B_{12}$, diabetic subjects with retinopathy excrete significantly more of the vitamin than the non-diabetics; while diabetics without retinopathy excrete less than the non-diabetics. Administration of testosterone to diabetics with retinopathy decreases the urinary excretion of a test dose of vitamin $B_{12}$.

### *Vitamin $B_{12}$ Serum Levels*[16]

Blood specimens were drawn from 28 subjects with retinopathy (9 males and 19 females) and from 26 subjects without retinopathy (8 males and 18 females), and sera were obtained by centrifugation and assayed for vitamin $B_{12}$ content. The results showed that the mean values of serum vitamin $B_{12}$ levels (with standard error of the mean) are 180.8 ± 14 and 294 ± 21 $\mu\mu$g/ml for the non-retinopathic and retinopathic groups, respectively. The difference is statistically significant and demonstrates an elevation of vitamin $B_{12}$ levels in patients with retinopathy.

### *Absorption of Vitamin $B_{12}$*[17]

To estimate the absorption of orally administered vitamin $B_{12}$ by diabetic subjects, urinary[18] and fecal excretion tests[19] were performed. In the first method, two $\mu$g of radioactive vitamin $B_{12}$ was administered by mouth to 15 diabetic patients with retinopathy, 13 without retinopathy and 65 nondiabetic subjects of a comparable age group. Two hours after the administration of the radioactive material, 1,000 $\mu$g of unlabeled vitamin $B_{12}$ was given intramuscularly to "flush" out the absorbed radioactive vitamin $B_{12}$. Twenty-four hour urine specimens were collected for the determination of radioactivity. It was found that the radioactivity in the urine of the diabetic subjects without retinopathy (6.25 ± 0.7%) was almost half that of the non-diabetic subjects (10.1 ± 0.8%). The difference is statistically significant. The diabetics with retinopathy excreted slightly higher amounts of radioactivity than the non-diabetics (11.3 ± 1.0%).

A more direct measurement was made by determining the fecal excretion of radioactivity following the administration of 1 $\mu$g of radioactive vitamin $B_{12}$ to the diabetic and healthy subjects.[17] Our data show that there was a greater amount of radioactivity in the feces of diabetic patients without retinal lesions (462 m$\mu$g) during eight days of collection than comparable specimens from non-diabetics (204 m$\mu$g) during eight days of collection. These data, therefore, lend support to the hypothesis that diabetics without retinopathy absorbed less of the orally administered vitamin $B_{12}$ than non-diabetics.

## DIABETICS AND THE ADRENAL CORTEX

Excellent reviews on this subject have been presented by Becker,[20] and they will not be repeated here. We would like, however, to emphasize a few basic points because they seem to us to have served as a great impetus to intensive research in this field.

### *Excretion of 17-Hydroxycorticoids and Retinopathy*

The late Dr. Friedenwald and his co-workers, as well as other investigators, pointed out that in diabetic subjects retinal lesions (capillary aneurysms) and renal (Kimmelstiel-Wilson) lesions are probably a manifestation of the same vascular disease process. On the basis of clinical, therapeutic, histopathologic and experimental evidence they advanced the hypothesis that the adrenal cortex is implicated in the pathogenesis of diabetic retinopathy with the Kimmelstiel-Wilson lesions. To test this hypothesis, a long series of experiments were undertaken. In an excellent study, Maengwyn-Davies[21] and her associates reported on the urinary 17-hydroxycorticosteroid excretion in 96 diabetic subjects with and without retinopathy compared to 39 non-diabetic controls.

Their data (Table VI) demonstrate that diabetic patients with retinopathy excrete a significantly larger amount of 17-hydroxycorticosteroids in 24 hours than diabetics without retinopathy, but there was no significant difference between either diabetic groups or normal controls. They also found that the diabetic group as a whole shows a significantly smaller urinary 17-hydroxycorticosteroid excretion after an oral test dose of compound F than do normal controls.

### *Xanthurenic Acid (Tryptophan) Excretion in Diabetics*

One of the consequences of hypertrophy of adrenal cortex may be greater requirements of vitamins such as pyridoxine.[22] Since diabetic subjects with retinopathy show some evidence of adrenal hypertrophy it would be of interest to determine whether they show any measurable signs of pyridoxine deficiency. Rosen[23] found that diabetic patients, irrespective of their retinal status, on the average excrete significantly greater quantities of xanthurenic acid after an oral test load of 10 g dl-tryptophan than do non-diabetic controls (Table VII). Table VIII shows the results obtained in six patients with diabetes in whom a *reversal* of high urinary excretion of xanthurenic acid was brought about by intramuscular injection of pyridoxine.

These data are interpreted to mean that many diabetic subjects are at least marginally deficient in pyridoxine. Whether this phenom-

TABLE VI

Comparison of 24-Hour Urinary Excretion of 17-Hydroxycorticosteroids by Normal Subjects and Diabetic Patients with and without Retinopathy*

| | Normals | | Diabetics | | | |
|---|---|---|---|---|---|---|
| | | | Without retinopathy | | With retinopathy | |
| | Female | Male | Female | Male | Female | Male |
| Number of cases | 16 | 23 | 28 | 19 | 32 | 17 |
| Age range | 20–66 | 17–84 | 32–73 | 27–74 | 35–73 | 36–76 |
| Range (mg) | 2.3–6.9 | 2.2–10.2 | 1.5–9.3 | 0.8–8.9 | 1.8–13.5 | 3.3–13.2 |
| Mean and standard deviation | 4.7 ± 1.55 | 6.5 ± 2.32 | 5.2 ± 1.93 | 4.7 ± 2.33 | 6.4 ± 2.29 | 6.6 ± 2.21 |
| Mean and standard deviation males and females pooled | 5.8 ± 2.22 | | 5.0 ± 2.04 | | 6.4 ± 2.21 | |

| | |
|---|---|
| Pooled retinopaths vs. non-retinopaths | $P < 0.01$ ($t = 3.42$) (18 cases) |
| Female retinopaths vs. female non-retinopaths | $P < 0.05$ ($t = 2.25$) |
| Male retinopaths vs. male non-retinopaths | $P < 0.05$ ($t = 2.47$) |
| Pooled retinopaths vs. pooled normals | $P > 0.05$ ($t = 1.42$) |
| Pooled non-retinopaths vs. pooled normals | $P > 0.05$ ($t = 1.78$) |

* Data reproduced from publication of Davies *et al.*[31] All values in mg of 17-hydroxycorticosteroids.

enon is due to hyperactivity of the adrenal cortex remains to be shown. Whether hyperactivity of the adrenal cortex in patients with diabetic retinopathy is pathogenetically related to diabetic retinopathy or is purely as associated phenomenon remains to be answered. The following animal experiments dealing with the interrelationships of vitamin $B_{12}$ and cortisone were initiated by Becker.

TABLE VII

Urinary Excretion of Xanthurenic Acid (mg in 24 hr) After Oral Test Load of 10 g *dl*-Tryptophan*

| Non-diabetics (1) | Diabetics without retinopathy (2) | Diabetics with retinopathy (3) |
|---|---|---|
| 12.02 | 26.86 | 32.02 |
| 0.00 | 54.23 | 47.52 |
| 3.83 | 12.70 | 19.12 |
| 10.78 | 41.20 | 41.63 |
| 13.25 | 27.95 | 54.02 |
| 20.35 | 17.58 | 84.26 |
| 4.36 | 13.59 | 24.60 |
| 13.48 | 6.06 | 15.36 |
| | | 29.70 |
| | | 41.20 |
| | | 45.90 |
| | | 23.35 |
| Mean | | |
| $\bar{X}_1$ 9.76 | $\bar{X}_2$ 25.02 | $\bar{X}_3$ 38.22 |
| $S\bar{X}_1$ 5.76 | $\bar{X}_{2,3}$ 32.94 | |
| | $\bar{X}_{2,3}$ 3.74 | |

$\bar{X}$ = mean; $S\bar{X}$ = standard error of mean.

* Data reproduced from a paper by Rosen *et al.*[23]

### *Vitamin $B_{12}$ and Cortisone in Rabbits*

It was mentioned previously that hyperactivity of the adrenals, or prolonged administration of cortisone results in hyperglycemia correctable with vitamin $B_{12}$. Becker and Friedenwald[24] reported that alloxan diabetes potentiated cortisone-induced lesions in rabbits. These lesions have definite similarities to the Kimmelstiel-Wilson nodules of the kidneys of

TABLE VIII

Reversal by Pyridoxine of Excess Urinary Excretion of Xanthurenic Acid (mg/24 hr) After Oral Test Load of 10 g *dl*-Tryptophan*

| Patient | Diabetic retinopathy | Xanthurenic acid | |
|---|---|---|---|
| | | Before pyridoxine | After pyridoxine |
| MFW | + | 35.00 | 24.58 |
| CW | − | 47.60 | 15.04 |
| AA | + | 36.36 | 27.20 |
| GH | − | 43.00 | 20.00 |
| ED | − | 37.80 | 18.20 |
| ML | − | 213.20 | 5.70 |

* Data reproduced from a paper by Rosen *et al.*[23]

the human diabetic subject. Since the diabetic state appears to be associated with a vitamin $B_{12}$ deficiency in the potentiation of the cortisone-induced renal lesions in rabbits, Becker and his associates performed the following studies.[24] They maintained one group (I) of albino male rabbits on a basal diet containing no added vitamin $B_{12}$ or Aureomycin. A control group (II) was placed on the same diet supplemented with vitamin $B_{12}$ (5.0 mg/ton) and Aureomycin (3.6 g/ton). A third group of rabbits like group I was fed the same basal diet, but received in addition 100 $\mu$g of vitamin $B_{12}$ daily by subcutaneous injection. Cortisone acetate (8.3 mg) was administered to all three groups daily for three weeks and at the end of this period, all animals were sacrificed and their kidneys were studied. It was found that of the animals maintained on the regular control diet 32 per cent revealed "cortisone-renal lesions." Of those maintained on the same diet, plus parenteral vitamin $B_{12}$ supplement, 33 per cent revealed similar lesions and in the group maintained on the experimental diet containing no added vitamin $B_{12}$, 100 per cent of the animals revealed "cortisone-renal lesions."

These results would suggest that the explanation for the observed potentiation of the "cortisone-renal lesion" by cortisone diabetes may be related to an associated vitamin $B_{12}$ deficiency.

It should be mentioned, however, that recently some doubt has been cast on the validity of the study just cited since it has been demonstrated that the rabbit has one of the highest blood levels of $B_{12}$ in the entire animal kingdom, and that it is almost impossible to produce a vitamin $B_{12}$ deficiency state in the rabbit. This may be due in a large measure to the fact that rabbits practice coprophagy.

## SUMMARY

This report consists of a brief summary of experimental studies which emphasize (a) the dominant role played by vitamin $B_{12}$ in the utilization of carbohydrates, (b) interrelationships between vitamin $B_{12}$ absorption and endocrine function, and (c) some relationships of vitamin $B_{12}$ to diabetes mellitus.

(1) Weanling rats fed a diet, rich in carbohydrate and low in fat require more vitamin $B_{12}$ for growth.

(2) The fat content of the carcass of vitamin $B_{12}$-deficient rats is abnormally low but can be elevated by injection of this vitamin.

(3) The administration of glucose to rats results in a decrease in the vitamin $B_{12}$ levels of plasma or liver.

(4) Deficiency of vitamin $B_{12}$ causes hyperglycemia correctable with vitamin $B_{12}$. *In vitro* experiments indicate that the red cells of vitamin $B_{12}$-deficient animals are low in glutathione content as well as enzyme activity essential for the degradation of glucose. Other enzyme systems involved in carbohydrate metabolism such as coenzyme A in liver or DPN in liver are also deranged due to this deficiency.

(5) The effect of cortisone and thyroid on absorption of orally administered vitamin $B_{12}$ and retention of injected vitamin $B_{12}$ was re-emphasized. It was found that corticotropin (ACTH) or cortisone causes hyperglycemia, destruction of vitamin $B_{12}$-binding substances in muscles as well as an elevation of the vitamin $B_{12}$ serum level. Thyroidectomy decreases absorption of vitamin $B_{12}$.

(6) Some evidence of adrenal hyperactivity in diabetic patients with diabetic retinopathy, (as gathered by other investigators), has been reviewed. We caution against a premature interpretation that adrenal hyperactivity is causally or pathogenetically related to diabetic retinopathy; it may turn out to be a mere associated phenomenon.

(7) Diabetic patients without retinopathy excrete in their urines a significantly smaller amount of an orally administered radioactive vitamin $B_{12}$-test dose than do non-diabetic subjects or diabetic patients with retinopathy. Fecal excretion tests confirm the above findings.

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# Goitrogenic Substances in Food

*By* MONTE A. GREER, M.D.*

ALTHOUGH it had been suggested for centuries that certain articles of diet might play a role in the development of nontoxic goiter, it was not until the work of Chesney, Clawson, and Webster, in 1928, that the first acceptable experimental evidence supporting this hypothesis was presented. These investigators found that a colony of rabbits they were maintaining for studies in experimental syphilis developed truly remarkable thyroid enlargement during their stay in the laboratory.[1–4] After examining other factors which might be involved, Chesney *et al.* came to the conclusion that the daily ration of cabbage was responsible. Although their studies indicated that the available iodine was more than adequate, additional supplementation of the diet with this element effectively prevented the development of goiter in the rabbits.

These studies were soon confirmed by various laboratories in diverse parts of the world. Marine was especially active in this field. Not only did he confirm the goitrogenicity of cabbage in rabbits, but also extended the original observations to include other related foods.[5]

A few investigators could not repeat the original findings of Chesney *et al.* Hercus and Purves,[6] working in New Zealand, were unable to produce any thyroid enlargement. They postulated, however, that the active material might be a glycoside and would therefore be expected to be contained in higher concentration in the seeds of the plants than in the edible parts. Accordingly, the New Zealand group made a study of the goitrogenic properties of various seeds of plants related to cabbage and found that a diet containing a high percentage of such seeds would regularly cause thyroid enlargement in rats.[6] Since these vegetables belong to the genus *Brassica*, the generic term "Brassica-seed goiter" has been subsequently applied in the literature to describe this phenomenon.

Subsequent studies by Purves and his collaborators[7–9] demonstrated that the thyroid enlargement appeared to be a compensatory hypertrophy. The Brassica-seed diet interferred with the normal formation of thyroid hormone, hypothyroidism resulted and secretion of thyrotropin by the pituitary was consequently increased. No goiter could be produced by this diet in hypophysectomized rats nor in intact rats administered thyroxin. One interesting and probably highly significant difference between their findings and the earlier studies of Chesney *et al.* with cabbage was that whereas cabbage-goiter could be prevented by added iodine, Brassica-seed goiter could not. Only a partial inhibition of thyroid hypertrophy would thus be produced.

Much thought and energy was expended on trying to elucidate the nature of the active goitrogen of the *Brassicae*. Marine originally considered that the active principle might be a mustard oil, since such compounds are characteristic for members of the cabbage family; however, none of those tried were found to be goitrogenic. Cyanide is also fairly prevalent among the *Brassicae* and all cyanides tested were found to produce thyroid enlargement. Methyl cyanide, the least toxic of this group, was most active.[10] He thus concluded that this compound was the active agent. Other investigators, however, have been unable to confirm the goitrogenicity of the cyanides.

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The next phase of the story develops from an entirely unexpected quarter. In 1941, Richter and Clisby,[11] testing various materials for their efficacy as rat poisons, noted that phenylthiourea would produce quite striking thyroid enlargement and hyperemia. At about the same time, Mackenzie, Mackenzie and McCollum[12] obtained similar effects in the rat during an investigation of sulfaguanidine. The next year, Kennedy, of the New Zealand group, reported that allylthiourea might be the active antithyroid compound in Brassica seed, since this compound was also goitrogenic.[13] Presumably he suggested allylthiourea because allylisothiocyanate is a common mustard oil among the *Brassicae* and isothiocyanates are known to form thioureas on reaction with ammonia.

These reports culminated in the careful and exhaustive studies of Astwood[14] into the chemical nature of antithyroid compounds. His investigations indicated that two classes of compounds interfered with the synthesis of thyroxin: the aniline derivatives, such as sulfaguanidine, and the thionamide series, such as thiouracil and thiourea. Only the thionamide series was known to be effective in man.

Although the studies of experimental goiter had greatly advanced our knowledge of thyroid physiology and factors which might be responsible for the development of goiter, it was hazardous to extrapolate the results of animal experiments to prove their possible significance in the etiology of human goiter. Isolated cases of the occurrence of thyroid enlargement in man due to the ingestion of goitrogenic foods had been reported from time to time, but the validity of these reports was subject to some question.

About 1946 an extremely important tool became available for the study of human thyroid physiology which had been denied earlier workers. This was the use of radioactive iodine. Utilizing a modification of the Stanley and Astwood rapid screening test[15] for the activity of antithyroid compounds in man by observing the acute change in slope produced by such materials on thyroidal $I^{131}$ accumulation, the antithyroid activity of a series of over 60 foods was studied.[16] (Table I). Several of these foods gave a positive test, but of these rutabaga was consistently most active. Contrary to our expectations, no activity could be detected in several tests of cabbage.

TABLE I

Results of Studies on Antithyroid Potency of Various Foods in Man (Modified from Reference)[16]

| Marked effect | Questionable effect | No effect | |
|---|---|---|---|
| Rutabaga | Grape | Beefsteak | Banana |
| Moderate effect: | Celery | Bonita | squash |
| | Green pepper | Cheese | Corn |
| Turnip | Orange | Ice cream | Rice |
| Peach | Apricot | Lobster | Rye |
| Pear | Peanut | Sardines | Black |
| Strawberry | Pea | Shrimp | beans |
| Spinach | String bean | Mushrooms | Lima beans |
| Carrot | Walnut | Dates | Onion |
| | Filbert | Pineapple | Olive |
| | Honeydew | Broccoli | Almond |
| | Cabbage | Cauliflower | Apple |
| | Lettuce | Mustard | Blackberry |
| | Beet | Radish | Loganberry |
| | Oyster | Cucumber | Tangerine |
| | Milk | | Banana |
| | Liver | | Potato |
| | Clam | | Tomato |
| | Grapefruit | | |

One interesting finding was that when the rutabaga had been cooked before eating, no activity could be found. If an aqueous extract of the cooked plant was subsequently treated with a purified enzyme preparation from uncooked rutabaga, it would acquire goitrogenic potency. This indicated that the active ingredient was initially present as inactive precursor and could be liberated only by specific enzymatic hydrolysis and not by the physiologic hydrolysis which would occur in the gut.

An investigation was then made into the nature of the antithyroid principle of rutabaga and related species. Eventually it was possible to isolate a colorless, crystalline material, "goitrin," which had high enough antithyroid activity to account for all the goitrogenic potency of rutabaga. It has been identified as L-5 vinyl, 2-thio-oxazolidone[17](Table II). This material has been found in rather high concentration in extracts from the seeds of

TABLE II

Thiooxazolidone Content of Seeds of Plants of Mustard Family[11]

| Genus | Plant | No. of varieties tested | Content | |
|---|---|---|---|---|
| | | | Range g/kg | Mean g/kg |
| Brassica | Rutabaga* | 7 | 0.8–8.6 | 2.5 |
| | Turnip* | 9 | 0.3–2.5 | 1.0 |
| | Cabbage* | 9 | 0.2–4.7 | 1.5 |
| | Kale* | 3 | 0.9–6.3 | 4.4 |
| | Rape* | 3 | 1.8–2.1 | 1.9 |
| | Chinese cabbage | 1 | | 0.7 |
| | Brussels sprouts | 2 | 0.5–0.8 | 0.7 |
| | Broccoli | 1 | | 1.6 |
| | Kohlrabi | 2 | 0.7–1.4 | 1.1 |
| | Cauliflower | 2 | | 0 |
| | Mustard | 3 | | 0 |
| Raphanus | Radish | 12 | | 0 |
| Lobularia | Sweet alyssum | 5 | | 0 |
| Matthiola | Stock | | | 0 |
| Iberis | Candytuft | 2 | | 0 |
| Nasturtium | Cress | 1 | | 0 |
| Lepidium | Cress | 1 | | 0 |
| Arabis | Rock cress | 1 | | 0 |
| Cheiranthus | Wallflower | 2 | | 0 |
| Lunaria | Honesty | 1 | | 0 |

* Identified by isolation.

most *Brassicae* and in the edible parts of rutabaga and turnip. Goitrin is slightly more active than propylthiouracil in man and about one fifth as active as propylthiouracil in the rat. It is in the thionamide series of antithyroid compounds and is closely related to methimazole.

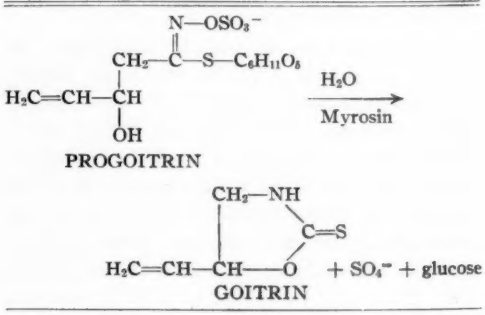
Recently it has been possible to isolate, crystallize, and identify the precursor of goitrin[18] (Table III). This compound has been named "progoitrin." It is a thioglycoside containing sulfate and glucose in addition to the goitrin portion which is present as the aglycone. Actually goitrin is not present in progoitrin as a cyclic structure but as a straight chain. Progoitrin is thus similar to other mustard oil glycosides, such as sinigrin. Because the aglycone contains an unstable hydroxyl group, it apparently cyclizes immediately after hydrolysis. With the elucidation of the correct structure of the mustard oil glycosides by Ettlinger and Lundeen,[19] it is now possible to write the correspondingly correct structure for progoitrin, rather than depending on the original structure for sinigrin proposed by Gadamer in 1897.[20]

Although several foods were found to possess antithyroid activity in our original studies, it has been possible to identify goitrin only in extracts of rutabaga and turnip. Eventually it may be possible to isolate the active compounds which may occur in other goitrogenic foods, but at present there is considerable question as to whether some of the positive effects we originally obtained may not have been artifacts. With rutabaga and turnip, of course, there can be no doubt. The content of progoitrin in the various Brassica seeds and plants varies from season to season and from batch to batch, even within identical varieties. The exact reason for this variation is unknown, but progoitrin has never been found completely absent in those varieties in which it has once been identified.

In view of the widespread occurrence of thioglycosides and the extensive studies being made of them at the present time, it will be quite surprising if other, and possibly more potent, active antithyroid compounds are not isolated from this series. As yet only the previously isolated 5,5-dimethyl, 2-thio-oxazolidone[21] has been found in hare's ear mustard. It is also quite likely that naturally occurring goitrogenic compounds of an entirely different

TABLE III[18]

Progoitrin Undergoes Specific Enzymatic Hydrolysis by the Thioglycosidase, Myrosin, to Form Goitrin by Cyclization of the Aglycone and Concomitantly Liberate Sulfate and Glucose



nature in a different family of foodstuffs will be discovered.

The failure to detect any goitrogenic activity in cabbage while finding it in cabbage seeds and other members of the *Brassicae* was quite surprising. It would indeed be odd if the plant which provided the initial stimulus for the massive accumulation of data on goitrogenic materials should prove to be completely impotent. Such may be the case, however. Complete prevention of cabbage-goiter by added iodine, as reported by Chesney *et al.* is not found either with Brassica-seed goiter or with the thyroid hypertrophy produced by pure antithyroid compounds. Also, the apparent production of hyperthyroidism by administration of iodine to cabbage-treated rabbits which had become goitrous is puzzling. If the goiter were due to the ingestion of compounds which interfere with the formation of thyroxine, it would not be expected that the goitrous thyroid would be capable of synthesizing thyroxine from the additional iodine. It is thus possible that the original cabbage-goiter may have been primarily due to iodine deficiency. It is also conceivable that the thiocyanate content of cabbage may have contributed by interfering with thyroidal iodide concentration without affecting organic binding of iodine by the thyroid.

Although certain cataclysmic events which necessitate a greatly increased consumption of goitrogenic foods by large population groups may contribute to an increased incidence of simple goiter,[22] it is exceedingly difficult to incriminate the ingestion of such foods as a factor in the etiology of the goiter in any individual patient. For the present, the best course would appear to be to make enquiries into the possible ingestion of such materials in each goitrous patient, but to concentrate our energies on other factors which may be involved. In any event, present evidence leads us to believe that goitrin cannot be formed from progoitrin if turnips and rutabagas are cooked before they are eaten. Only the consumption of raw vegetables of this class should therefore be looked upon with suspicion.


## SUMMARY

The more recent literature on the production of goiter by various foodstuffs is briefly reviewed. Rutabaga and turnip are the only edible plants from which an active goitrogen has so far been isolated. The active antithyroid agent in these vegetables has been identified as goitrin. It is present in the plant, and in the seeds of most *Brassicae*, as progoitrin, an inactive compound. Goitrin is apparently liberated from progoitrin only through specific enzymatic hydrolysis by a thioglycosidase contained in the plant or seed itself. Cooking destroys this enzyme and thus negates its goitrogenic potency by preventing the liberation of goitrin from progoitrin. Although the ingestion of considerable quantities of goitrogenic foods may contribute to the development of goiter under certain circumstances, it is very difficult to incriminate them as an etiologic factor in the vast majority of goitrous patients.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# The Influence of the Hypophysis and Adrenals on Digestive Function

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NUMEROUS important clinical observations have been reported on the changes in digestion which accompany various diseases of the endocrine glands. But investigators in basic endocrinology have given rather little attention to evaluation of gastrointestinal competency under experimental conditions. Too often loss of appetite and reduction in food intake arising from drastic hormonal therapy pass unnoticed. The seriousness of this oversight becomes obvious when one realizes that hypofunction of the adrenal cortex, thyroid or islands of Langerhans, or administration of excessive amounts of certain hormones elaborated by these glands, will result in general retardation in the rate of body growth. More complete knowledge concerning the role played by hormones in maintaining the structure and regulating the function of the digestive tract should aid greatly in evaluating the metabolic changes elicited by hormones.

The hormones which affect gastrointestinal activity fall into two general categories: (a) those which are elaborated intrinsically within the digestive tract and (b) those which are secreted extrinsically by the glands of internal secretion. The intrinsic group includes gastrin, secretin, cholecystokinin and pancreozymin. In this presentation we are concerned with the extrinsic hormones exclusive of neurohumoral substances. We shall attempt to correlate the morphologic and functional changes which occur in the digestive tract when the level of hypophyseal, adrenocortical, thyroid, or gonadal hormones is elevated or lowered.

For purposes of discussion, gastrointestinal function may be divided into the following aspects: (a) motility of the gastrointestinal wall, (b) secretion into the lumen of mucus, various ions, fluid and enzymes, (c) the enzymatic break-down of nutrient materials within the lumen and (d) absorption of the products of digestion.

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## MOTILITY

Except for the neurohormones, little information is available concerning the action of the extrinsically-produced hormones on gastrointestinal motility. Following hypophysectomy, the digestive tract exhibits a considerable loss in weight.[1] Although the mucosa is involved,[2] nothing is known concerning structural alteration in smooth muscle. Gastric emptying time is retarded by hypophysectomy of dogs.[3] Adrenalectomy usually results in increased motility by the small intestine of rats.[4] As is to be expected, direct action on enzymatic processes within the lumen of the gut have not been demonstrated.

## ABSORPTION

Normal absorption is clearly dependent upon the action of extrinsic hormones. Hypophysectomy slows the absorptive rate for glucose, fat and protein.[5,6] It is possible that these deficiencies arise because of the impaired functional capacity of the adrenal cortex and thyroid. Adrenalectomy retards the absorption of fat,[7,8] sodium chloride[9] and glucose.[10] Maintenance of the animal on salt may[10] or may

not[11] restore to normal the absorptive rate for glucose. It does not elevate the depressed absorption of fat.[8] Althausen[12] concluded that diminished absorption of glucose after adrenalectomy is due either to general impairment of health or disturbed electrolyte metabolism. The administration of high doses of thyroxine (0.10–0.02 mg)[13] (0.1 mg)[12] to hypophysectomized rats restores the absorptive rate for glucose to normal. Doses of this magnitude are far beyond the replacement level and lower doses are ineffective.[12] Therefore, the restoration in rate of absorption may be the indirect result of increased metabolic requirements of the hyperthyroid state although the suggestion has been made that the original deficiency involves defective phosphorylation by the intestinal epithelium.[11,12]

Following hypophysectomy, the weight of the gastrointestinal mucosa is reduced.[2] The chief anatomic structure upon which absorption depends is the intestinal villus. It has received little attention in states of endocrine dysfunction. In hypophysectomized birds its height is reduced significantly below normal.[1] A similar change occurs in our hypophysectomized rats. The villous epithelium is modified histochemically but there is no profound atrophy of its cells. Retardation occurs in the rate of renewal of the epithelial cells,[14] which normally proliferate at the base of the villus and move apically to be extruded at the tip.[15] Adrenalectomy elicits a reduction in alkaline glycerophosphatase[16,17,18] and adenosinetriphosphatase[19] activities in the intestinal epithelium. Administration of adrenocortical hormones is exceedingly effective in hastening the time of appearance of phosphatase in the nursing mouse,[20] and regenerating it in adrenalectomized animals.[11] The relationship of the depression in alkaline phosphatase to the lowered absorption of glucose is not clear.

Some of the anatomic changes which occur in the intestinal villus during various endocrine states of hyper- or hypofunction are significant but by themselves do not appear sufficiently great to account for the defective absorption which is present. Therefore, one should consider whether the condition of the intra-luminal milieu is favorable to the digestive process. Light may be thrown on this problem by observation of the secretory capacity of the gastrointestinal mucosa using morphologic and biochemical methods. Considerable information is available concerning the influence of hormones on the elaboration and release of secretory products. In the subsequent attempt to synthesize present information regarding hormonal regulation of secretory processes, confusion will be avoided if the role played by hormones in maintaining the structural and chemical capacity to synthesize secretions is differentiated from their action as direct stimulators of release of the secretory product. It is recognized that in many instances a sharp differentiation between these two actions on a cell may be impossible. Thus, an agent which induces release of a substance from the cell may at the same time stimulate its synthesis with the accompanying structural manifestations of this process.

## SECRETION

*Mucus:* Little is known of the action of hormones on the production of mucus. After adrenalectomy, Baker and Bridgman[16] observed an irregular reduction in the amount of mucus in epithelium of the pits and mucosal surface of the rat stomach and in intestinal goblet cells. Since a secretory stimulant caused the release of more mucus from the stomach of adrenalectomized than of non-adrenalectomized rats,[21] the lower amount which we observed to be stored after ablation of the adrenals may have been associated with augmented release rather than inhibited synthesis. In general the cytology of mucus-producing cells is resistant to alteration by hormonal action.[22]

*Hydrochloric Acid:* Following hypophysectomy of rats[23] and dogs,[3] the volume and total acid of gastric juice is reduced and its pH elevated. The cellular source of hydrochloric acid, the parietal cell, exhibits parallel involutional changes.[24] A depletion of intracellular fuchsinophilic granules occurs but unfortunately the relationship of these granules to the secretion of hydrochloric acid is unknown. Friedman[25] reported a 50 per cent reduction in the number of parietal cells following hypophysectomy, but this has not appeared to be the case in our animals.

The manner in which the hypophysis modifies parietal cells is not clear. Although the lowered free acid of Addison's disease is well-demonstrated, the influence of adrenocortical deficiency in animals is less clear-cut. There is uniform agreement that adrenalectomy reduces the volume of gastric secretion.[21,26,27,28] Although a marked fall in free and total acid was observed in the Shay rat,[28] Madden and Ramsburg[26] found no change in pH. *In vivo* administration of corticotropin increases the secretion of free hydrochloric acid,[29] but since neither cortisone nor desoxycorticosterone affects gastric secretion directly *in vitro*,[30] it may be assumed that the adrenal cortex influences the gastric mucosa through some intermediary mechanism. The influence of the thyroid is less clear although Sun *et al.*[31] detected a lowered free and total acid concentration eight weeks after thyroparathyroidectomy, administration of radioactive iodine or of thiouracil. These workers suggest that the thyroid influences gastric secretion through the adrenal cortex.

*Enzymes:* For the past several years a systematic study of the zymogenic cells has been pursued in this laboratory in order to determine the extent of hormonal control over them and the manner in which it is exerted. All types of cells associated with the digestive tract which are known to produce one or more enzymes exhibit varying degrees of involution after pituitary ablation. Many supplementary experiments involving excision of other endocrine glands and replacement therapy of hypophysectomized rats with various hormones have been carried out in an attempt to define precisely the pathways of hormonal control. A summary of the results of these experiments and of the hormonal patterns which appear to be operative will be presented.

*Gastric Chief Cells:* Following hypophysectomy a prompt reduction in size of chief cells occurs with loss of pepsinogen granules and cytoplasmic ribonucleic acid.[24] By the seventh postoperative day peptic activity in the gastric juice of hypophysectomized, pylorus-ligated rats is 80 per cent less than that of control animals.

Somewhat similar structural change follows adrenalectomy[16] and is accompanied by an impaired capacity to secrete pepsinogen.[21,27] No alteration in structure of chief cells follows ovariectomy. Moderate and variable involutional changes occur after thyroidectomy (Baker, unpublished) if the postoperative period is prolonged. However, these experiments are open to criticism on the grounds that a state of complete thyroid deficiency may not have been produced.

Although administration of adrenocortical extract to adrenalectomized rats restores the secretion of pepsinogen to normal,[21] this goal has not been achieved in hypophysectomized rats. A wide variety of hormones have been administered to hypophysectomized rats including cortisone acetate, cortisol, corticosterone, thyroxine, desiccated thyroid, somato-

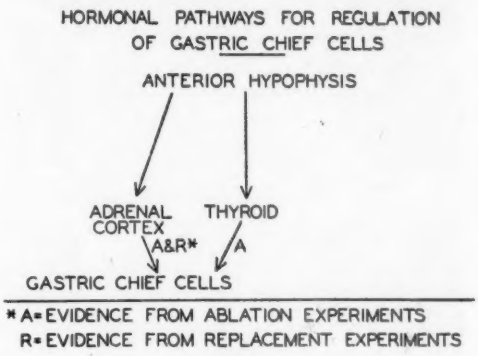


Fig. 1

tropin, thyrotropin, lactogen, and corticotropin, singly and in various combinations.[22,32] If involution had been permitted to occur after hypophysectomy before the initiation of therapy, none of these treatments would have restored to normal the structure or zymogenic granulation of the chief cells. Initiation of therapy with cortisol at the time of hypophysectomy partially prevented the involution of chief cells.[22] Full restoration was effected only by implantation of whole pituitary glands.[32] Unfortunately, this type of experiment fails to reveal which pituitary factor(s) is responsible for recovery.

Involvement of the thyroid in the induction of digestive deficiency after hypophysectomy is indicated by the mild fall in the total amount of pepsin secreted by thyroid-deficient Shay

rats[27,31] and by a moderate involution of the chief cells after a prolonged post-thyroidectomy interval. Tentatively, it may be concluded that maintenance of secretory competence is effected by the hypophysis through the adrenal cortex, with some participation by the thyroid (Fig. 1). The failure of adrenocortical hormones to restore the structure of chief cells in hypophysectomized rats indicates clearly the participation of other hormones in the regulation of these cells.

Adrenocortical hormones are important factors in stimulating the release of pepsinogen by chief cells. Administration of corticotropin or cortisone to man increases the peptic activity of gastric juice and the excretion of uropepsin;[33] corticotropin also augments the gastric peptic activity of dogs.[34] Therapy of hypophysectomized rats with cortisone acetate for a week increases the secretion of pepsin.[22] In view of the atrophic state of the chief cells in hypophysectomized rats and failure of cortisone therapy to restore their structure, the elevation of pepsinogen secretion by this treatment is a remarkable outcome. Cytologic evidence of stimulation of chief cells by cortisone acetate or cortisol has been observed also in normal rats.[16]

*Pancreas:* Hypophysectomy induces a profound involution of the pancreas which involves the exocrine or zymogenic portion. Thirty-five days after the operation in rats, the weight is 60 per cent less than in normal rats.[22] As would be expected, this is a reflection of cytologic involution of the enzyme-producing cells. Although the degree of change varies in different parts of the gland, in the lobules which are affected most severely the cells are small, depleted of zymogenic granules and ribonucleic acid and possess smaller nuclei[32,35] (Fig. 2). As determined by biochemical analysis of homogenates of the gland, the amount of amylase,[35,36] proteinase[37] and lipase[38] contained within the gland is reduced significantly. Considering the effect of excision of other glands controlled by the anterior hypophysis, including the adrenals, testes and thyroid, only removal of the latter reduces the weight of the pancreas to a degree which approximates the magnitude of change elicited by hypophysectomy.[39]

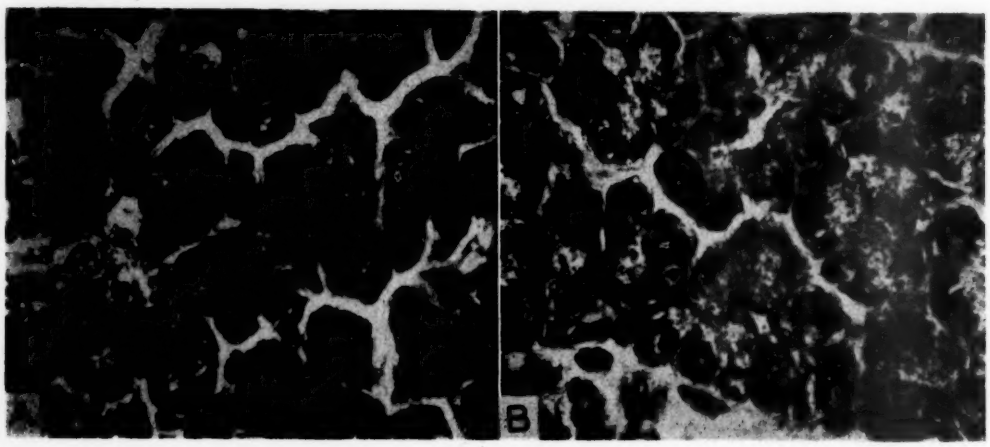


Fig. 2. (A) A section of pancreas from a non-hypophysectomized control animal. (B) The pancreas of a rat which was hypophysectomized 35 days previously. Bouin fixation, chrome alum hematoxylin and phloxine stain.

Treatment of hypophysectomized rats with thyroxine restores the concentration of amylase,[39] and daily implantation of pituitary glands brings the weight back to a nearly normal level.[32] Of the many other hormones which have been used, only cortisone acetate[22] and prolonged treatment with somatotropin[40] have increased pancreatic weight in hypophysectomized rats. Thus, the limited available evidence indicates clearly that the secretory

capacity of the exocrine pancreas is influenced mainly by the anterior hypophysis through the thyroid (Fig. 3). The capacity of the extrinsic hormones to induce release of enzymes apart from maintenance of the cells has not been investigated.

*Parotid:* Following hypophysectomy, the parotid weighs less, its zymogenic cells are smaller and contain fewer zymogenic granules than in control animals.[22] It should be emphasized that the intensity of these cytologic alterations does not approximate that which occurs in the gastric chief cells and pancreatic acini, where the reduction in cellular size, granular content and ribonucleic acid is much

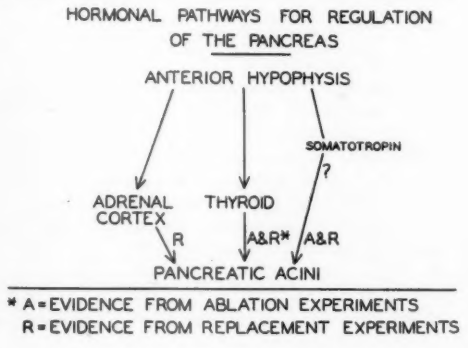


Fig. 3

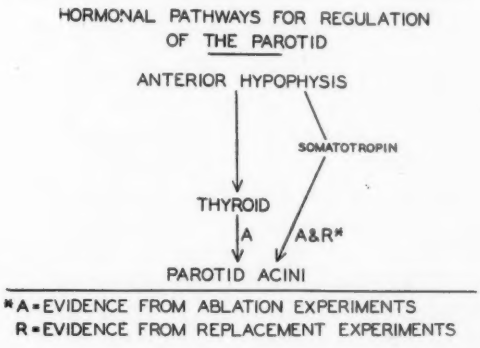


Fig. 4

greater. Accompanying the partial involution of the parotid is a profound reduction in the amount of amylase which it contains.[41]

Much interest in the parotid has been created by the postulation by Japanese investigators[42] that pituitary growth hormone exerts its peripheral effects through the parotid, which in turn is believed to secrete a hormone, parotin. This theory underlines the need for additional studies to determine the manner in which the hypophysis influences the parotid. Following ablation of the adrenals, thyroid or gonads, singly or in combination, a fall in weight of the parotid occurs only if the thyroid is ablated, albeit this is of less magnitude than that which follows hypophysectomy.[32] No treatment with pure or purified hormones has been found which will augment the weight of the parotid in hypophysectomized rats other than with somatotropin[22] or some combination of hormones which contains this factor. When somatotropin is given in 1 mg daily doses for seven days beginning 10 to 14 days after hypophysectomy, the absolute weight of the gland is increased but not out of proportion to the concurrent elevation in body weight. With implantation of pituitary glands into hypophysectomized rats, a profound stimulation of the parotid is induced. Weight of the gland in hypophysectomized rats is increased 51 per cent above the level for *non-hypophysectomized controls* which did not receive the implants.[32] Areas of glandular atrophy and replacement with fat are frequent in such glands. The immediate cause of this atrophy is not apparent but it is of a type which might follow hypersecretion and blockage of ducts. These results indicate clearly that the hypophysis contains some factor(s) capable of intense stimulation of the parotid.

It may be inferred tentatively that the thyroid plays a part in pituitary regulation of the parotid and that growth hormone may be involved (Fig. 4). Final acceptance of the Japanese hypothesis is not possible at this time, chiefly because interference with growth after sialo-adenectomy is of such a minor degree.[43,44] If pituitary somatotropin were dependent on a functional parotid for its action, the degree of interference with growth after excision of the salivary glands should approximate that which follows hypophysectomy.

## DISCUSSION

In addition to the zymogenic cellular types discussed previously, equally profound changes occur in the tubular zymogenic cells of the rodent submandibular gland.[22] Paneth cells

of the intestine are altered similarly although they have not yet received intensive study.

These observations make clear that the pituitary gland influences many aspects of digestive function. Probably the most profound effect is on the enzyme-producing cells. The considerable magnitude of their involution after hypophysectomy indicates the existence of direct hormonal action, either by a pituitary hormone, a hormone elaborated by a ductless gland which is regulated by the hypophysis, or by a combination of these humoral factors. This can be proven conclusively only by complete restoration of the cells in the absence of the pituitary gland. To date, full restoration by replacement therapy in the absence of the hypophysis has not been accomplished for any type of zymogenic cell. This difficulty in itself suggests that the partial involution of zymogenic cells in the hypophysectomized animal may be an adaptation to a lower level of general metabolism.

Regardless of our current inability to define conclusively the pathways for hormonal support of zymogenic cells, there are some definite danger signals in these observations which should be heeded by endocrine investigators. It is almost certain that any hormone studied in a hypophysectomized (and in some cases an adrenalectomized or thyroidectomized) animal will be acting against a background of digestive deficiency. Our experience teaches us that many hormones may induce significant structural and functional changes in peripheral tissues of hypophysectomized animals, yet they fail to repair all of the digestive deficiencies. Thus when growth is a part of the peripheral response to a hormone (as sex hormones acting on the reproductive system), the capacity of tissues to respond may well be limited by failure of the digestive system to meet fully the nutritional requirements of the activated peripheral tissue.

## CONCLUSION

Hormones produced outside of the gastrointestinal tract modify several aspects of alimentary function. In particular the efficiency of absorption and of enzyme production is dependent on hormonal support. All of the enzyme-producing cells which have been studied exhibit involution and functional depression in the absence of the pituitary gland. This applies to the gastric chief cells, exocrine pancreas, parotid gland, zymogenic cells of the submandibular gland and intestinal Paneth cells. It is probable that their deficiency accounts in some measure for the impaired absorption of nutrients after removal of the hypophysis. The manner in which the pituitary gland regulates zymogenic cells has not been demonstrated conclusively but the hormonal pattern appears to be distinctive for each cell type. The adrenal cortex mediates some of the pituitary action on the gastric chief cells, and the thyroid, that on the pancreas.

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# Influence of the Pituitary on Hemopoiesis

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THE HYPOPHYSIS does, indeed, have an influence upon hemopoiesis. It is our feeling, however, that many readers are quite familiar with the fact that corticotropin (ACTH) and adrenal hormones have an effect on peripheral white-cell elements; we would like, therefore, to concentrate, for the most part, upon the effect of the hypophysis on the development of the erythrocyte—erythropoiesis.

Since the monumental work of Aschner[1] in 1912 it has been known that the pituitary gland has an influence on this process. The anemia in hypophysectomized dogs reported by Aschner, however, was ignored for many years. In the late thirties and early forties several papers were published in rapid succession showing that this anemia could be demonstrated in other laboratory animals. It has been shown to be present in every hypophysectomized animal investigated, and in the human being with panhypopituitarism.

The early work in this field consisted mainly in finding a therapy which would either prevent or cure this anemia which came to be called post-hypophysectomy anemia.[2] This anemia is characterized by significant decreases in erythrocyte count, hematocrit, and hemoglobin; it is slightly microcytic-hypochromic or normocytic-normochromic in type, depending on the strain of rats; and it is accompanied by a decrease in erythroid elements in the bone marrow.

Figure 1 summarizes results obtained in our laboratory on the erythrocyte count in hypophysectomized rats treated by various therapeutic means. It is easily seen that iron or iron and copper were beneficial but did not prevent the anemia; that thyroxine or androgen, whether given alone or in combination with other substances, were quite effective. Figure 2 shows, however, that these substances were not as effective in preventing the decrease in hemoglobin induced by hypophysectomy, a

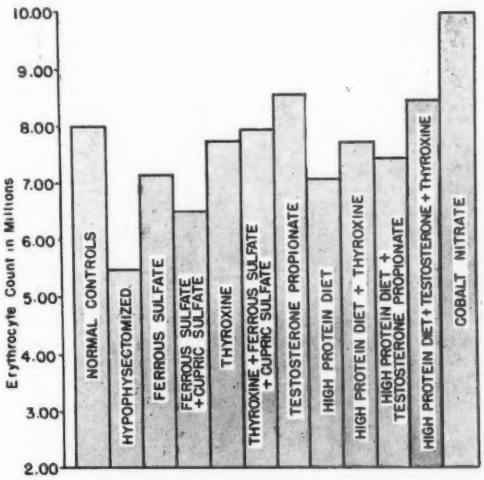


Fig. 1. A comparison of the effects of various therapies on the erythrocyte count of hypophysectomized adult female rats. All figures are after 40 days of treatment. (Taken from Crafts, R. C. *Blood* 7: 863, 1952.)

combination of thyroxine, testosterone propionate, and a high-protein diet being the only therapy which was entirely effective.[2] Subsequent work[3] comparing the effectiveness of thyroxine plus testosterone propionate with results obtained with these same hormones together with a high-protein diet showed that the high-protein diet was effective; the rats receiving the high-protein diet exhibited higher

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counts than those receiving the hormones with a regular diet.

Cobalt nitrate induces an amazing response in the blood picture of hypophysectomized animals[4,5]; cobalt, injected for 50 days, not only prevented post-hypophysectomy anemia but induced increases in erythrocyte count from a normal of eight million cells per cu mm to twelve million cells; the hemoglobin increased from 15.8 g per 100 ml to 21.0 g.

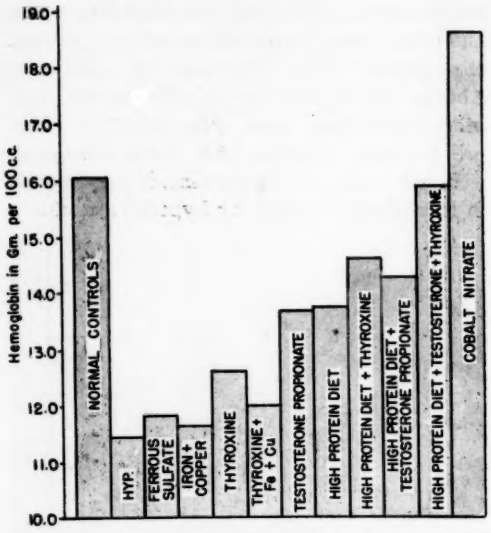


Fig. 2. A comparison of the effects of various therapies on the hemoglobin of hypophysectomized adult female rats. All figures are after 40 days of treatment. (Taken from Crafts, R. C. *Blood* 7: 863, 1952.)

Because of the finding that vitamin $B_{12}$ contained cobalt, some hypophysectomized rats were injected with this vitamin and others with liver extract. Neither of these substances had an effect on post-hypophysectomy anemia. The mechanism by which cobalt stimulates erythropoiesis in these rats is still unknown.

### THYROID AND ADRENAL CORTEX

In 1938 Flaks, Himmel and Zlotnik[6] made an extract of cattle pituitary glands, fed it to hypophysectomized rats and reported that erythropoiesis was stimulated; they claimed the presence of an erythropoietic "hormone" or "factor" in the anterior lobe of the hypophysis. Contopoulos and co-workers,[7] repeated this work and, although they could not find this factor in cattle pituitary glands, they did find it in sheep glands.[7] Two more reports followed this discovery[8,9] also claiming the presence of a pituitary erythropoietic factor. In all reports the pituitary extract was given orally. We have commented several times[3,10,11] on the fact that it is strange that a pituitary hormone should be effective when given orally when other known hormones from this gland are not.

In addition, we reported[10] that combined thyroidectomy and adrenalectomy induced a peripheral anemia similar to that following removal of the hypophysis in spite of the fact that thyroidectomy alone induces a mild anemia[12–14] and adrenalectomy a temporary decrease in erythrocytes.[12,15,16] We also showed that combined thyroxine and cortisone therapy would prevent post-hypophysectomy anemia,[11] a fact recently substantiated by accomplishing the more difficult feat of eliminating the anemia in hypophysectomized rats after it had become established with the same amounts of thyroxine and cortisone.[17] This series of experiments resulted in one school of thought which claimed that post-hypophysectomy anemia was due to a loss of a special pituitary erythropoietic factor, and another which indicated that the anemia was simply due to the hypothyroidism and hypoadrenocorticalism which inevitably follow hypophysectomy.

This stimulated Contopoulos and co-workers[9] to repeat this work and they found that combined thyroidectomy, adrenalectomy, and orchidectomy induced an anemia but one which was not as severe as that following hypophysectomy. These workers used Long-Evans rats while ours had been of the Wistar strain. Thinking that the strain difference might have been responsible for this discrepancy, a project was undertaken comparing the effects of hypophysectomy and of a combined thyroidectomy and adrenalectomy on the blood picture in adult female rats of the Long-Evans strain, the Wistar strain, and a third strain—the Sprague-Dawley.[18] Once again we found that the peripheral anemia after either surgical procedures was similar. However, utilizing a slight mod-

ification of a method introduced by Fruhman and Gordon[19] for determining actual counts of marrow cells per cu mm of marrow tissue, we found that the anemia following thyroidectomy and adrenalectomy did not have exactly the same features as that following hypophysectomy.

The hypophysectomized animals exhibited an anemia with the following features: (1) a decrease in erythrocyte count, hematocrit reading, and hemoglobin level; (2) a type characterized as slightly microcytic-hypochromic; and (3) a bone marrow showing a decrease in erythroid elements, no significant changes in myeloid elements, and a marked increase in fat. Combined thyroidectomy and adrenalectomy induced an anemia with the following characteristics: (1) a decrease in erythrocyte count, hematocrit, and hemoglobin similar to that found after hypophysectomy; (2) a normocytic-normochromic type; and (3) a bone marrow with a less severe decrease in erythroid elements than found after hypophysectomy, an increase in myeloid elements, and no increase in fat. In the meantime, Contopoulos *et al.*[20] gave their erythropoietic factor by subcutaneous injection and reported a polycythemia; in addition, they were able to prevent neonatal anemia by intraperitoneal injections of this same factor.[21]

In spite of these findings, we still felt that the thyroid and adrenal glands were involved in post-hypophysectomy anemia. In an attempt to solve this discrepancy Dr. Contopoulos very generously sent us some of his erythropoietic factor. We tested this material according to our methods using hypophysectomized adult rats of the Wistar strain. This material, given as he instructed, was ineffective; it had no effect upon hemopoiesis in our hypophysectomized rats. It did induce a slight but significant increase in adrenal weights. We cannot explain these results other than to note that Contopoulos *et al.*[22] have reported that their extract increased basal metabolic rate in hypophysectomized rats, and even in thyroidectomized rats. We will show you later that the extract in our hands did not increase oxygen consumption in hypophysectomized animals. Dr. Contopoulos has promised to send us more of this hormone, and, until he does, we can do nothing but remain skeptical.

We have to admit, however, that hypothyroidism and hypoadrenocorticalism will not entirely explain post-hypophysectomy anemia. The pituitary seems to secrete something that effects erythropoiesis besides thyrotropic and adrenocorticotropic hormones. Growth hormone cannot be ignored. Although six separate investigations[23–28] have shown that this hormone induces no changes in the peripheral blood picture of hypophysectomized rats, Fruhman, Gerstner and Gordon[28] claimed that this was due to a hemodilution which accompanies growth-hormone therapy. To support this theory, they presented an increase in reticulocytes and a hyperplastic bone marrow in their growth-hormone-treated rats. To complicate the picture even further, Querido and Overbeek[29] and Overbeek[30] found that growth hormone had no influence on the reticulocyte count.

### GROWTH HORMONE

We have made a detailed study of the effects of growth hormone on hemopoiesis in the hypophysectomized rat[31] and found that it produced a hyperplasia of the bone marrow, the erythroid elements responding in a more marked fashion than the myeloid; these changes were accompanied by an increase in reticulocyte percentage. In spite of this growth of the marrow, the peripheral anemia was not altered. Blood volume studies did not support the view that this lack of effect on the peripheral blood was due to a hemodilution, a finding which is in agreement with Van Dyke *et al.*[25] In another paper published by Drs. Fruhman and Gordon,[32] these workers again conclude that growth hormone stimulates erythropoiesis in hypophysectomized rats and that "the failure of the peripheral values to rise in these rats is attributable to the concomitant increase in plasma volume." The authors, however, have used the "initial" body weight of the animals in calculating red cell volume rather than the weight of the rats at the time when the plasma volumes were actually determined. We consider this pro-

cedure to be open to question. We explain our positive results on reticulocyte count and on bone marrows and our negative results on the peripheral blood of hypophysectomized rats treated with growth hormone by taking the increase in size of the rat into consideration. We feel that growth hormone allows the hypophysectomized rat to produce enough extra blood to accompany its increase in size. This agrees with the findings of Gemzell and Sjöstrand[27] who measured the total amount of hemoglobin in untreated hypophysectomized rats and in hypophysectomized rats treated with growth hormone; the total hemoglobin was increased in the treated rats but only to a degree which corresponded with the increase in body weight of the animals.

TABLE I

Blood Picture and Oxygen Consumption in Hypophysectomized Adult Female Rats before and after a Blood Transfusion. Average Amount of Blood Transfused was 4.5 ml. ± = Standard Error

| | Normal controls | Hypophysectomized rats before transfusion | | Hypophysectomized rats after transfusion | |
|---|---|---|---|---|---|
| | 13 Rats | 7 Rats | % of Normal | 7 Rats | % of Normal |
| Erythrocyte count in millions per cu mm | 8.21 ±0.16 | 6.99 ±0.17 | 86 | 9.39 ±0.36 | 114 |
| Hematocrit in per cent | 46.3 ±0.75 | 36.3 ±1.10 | 79 | 48.1 ±1.60 | 103 |
| Hemoglobin in g per 100 ml | 14.9 ±0.23 | 13.2 ±0.28 | 89 | 16.3 ±0.46 | 109 |
| Oxygen consumption in liters per square meter body surface per hour | 7.79 ±0.22 | 5.26 ±0.17 | 68 | 4.71 ±0.24 | 61 |

Summarizing this part of the work, we believe that hypothyroidism and hypoadrenocorticalism are involved in post-hypophysectomy anemia, and that the pituitary is involved in some manner in addition to its role of stimulating the thyroid and adrenal glands. What is this additional factor? We do not have much faith in the pituitary erythropoietic factor; this additional factor may be growth hormone. At the present time we are treating hypophysectomized rats with thyroxine, cortisone, and growth hormone in an attempt to produce a real polycythemia.

## ETIOLOGY

We would like to theorize about the actual cause of post-hypophysectomy anemia. Although we have seen that removal of various glands will induce anemias, and that various therapeutic substances will prevent this anemia if injected immediately after hypophysectomy, or restore a normal blood picture if injected after the anemia has developed, we do not believe that these effects are due to direct control of the bone marrow by the endocrine glands. We have stated repeatedly that these effects are probably mediated through the role these glands play in metabolism.[2-4,10-12,17,18,26,31,33,34] With the exception of cobalt, all of the substances which proved beneficial have been those capable of affecting metabolism in one way or another.

We would like to go one step further and theorize that the actual mechanism concerned in this anemia is the need for oxygen by the tissues, a step which takes us back to the well-known and still important fundamental stimulus to erythropoiesis, namely anoxic anoxia. We believe that the hypophysectomized animal is anemic because these animals are existing at a decreased tempo, so to speak, which in turn decreases the demand of the tissues for oxygen; erythropoiesis, therefore, slows down, for after all, the function of the erythrocyte and its contained hemoglobin is oxygen and carbon dioxide transport.

If the pituitary gland controls erythropoiesis directly, as is suggested by sponsors of a pituitary erythropoietic factor, erythropoiesis

should cease upon removal of the gland. This is not the case; the bone marrow in hypophysectomized animals is capable of normal functions as can be shown in several ways. (1) The erythrocyte count, hematocrit and hemoglobin levels in hypophysectomized rats do not show a continued decrease; approximately 40 days after hypophysectomy the blood picture ceases to change and levels off, the number of erythrocytes fluctuating around the same level for as long as a year after hypophysectomy. (2) Although Meyer *et al.*[23] could not induce responses in the bone marrow of hypophysectomized rats by decreasing the $O_2$ tension, Feigin and Gordon[35] were able to do so by subjecting the animals to a more severe decrease in $O_2$ tension. (3) Silbergleit,[36] in my laboratory, found that the hypophysectomized rat responded to hemorrhage by an erythropoiesis, the blood returning to a level typical of hypophysectomized rats only. (4) Baker *et al.*[37] have shown that the hypophysectomized rat treated with total irradiation with x-ray will become more anemic than untreated hypophysectomized rats, but will recover to such an extent that the blood picture is typical of the untreated hypophysectomized animals. (5) In addition, the remarkable response of the hypophysectomized rat to cobalt should not be forgotten. Therefore, the hypophysectomized rat has marrow tissue which will function properly in the absence of the pituitary gland.

### OBSERVATION ON OXYGEN

If this "oxygen need" theory is correct, oxygen consumption in the hypophysectomized rat should be decreased. This has been shown to be true several times[38–40] and the data in Table I add further proof on this point. Table I also shows the results of an experiment done to determine whether this decrease in oxygen consumption was due to actual decreased tissue consumption or caused by a lack of erythrocytes for transport purposes. Adult female rats were hypophysectomized and left unmolested for 60 days. At this time a single rat was anesthetized with ether, blood was removed from the tail for analysis, and an oxygen consumption was determined. Hypophysectomy had induced an anemia and the oxygen consumption was 5.26 liters per hour in contrast to a normal value of 7.79 liters. On the following day these rats were given a transfusion of an average of 4.5 ml of normal whole blood via tail vein. This was followed by an oxygen consumption determination, and then blood was removed from the tail for analysis. The erythrocyte count, hematocrit reading, and hemoglobin values were restored to normal by the transfusion. In spite of this, the oxygen consumption remained at a lower than normal level which indicated that the decreased oxygen consumption was due to a decreased utilization by the tissues rather than to lack of a transport mechanism.

If decreased oxygen need at the tissue level is the actual explanation, the thyroidectomized-adrenalectomized rats which were anemic should show a decreased oxygen consumption and treatment which proved to be beneficial should cause an elevation of oxygen consumption at the same time as improvement in the blood picture is obtained; conversely, those measures which had no effect on the blood picture should have no effect upon oxygen consumption. Table II shows that the dual operation of thyroidectomy and adrenalectomy does indeed induce a decrease in oxygen consumption.

TABLE II

Effects of a Combined Thyroidectomy and Adrenalectomy on the Blood Picture and Oxygen Consumption of Adult Female Rats. Studies Made 55 Days after Operation. ± = Standard Error

| | Normal controls | Thyroidect. and adrenalect. | |
|---|---|---|---|
| | 9 Rats | 6 Rats | % of Normal |
| Erythrocyte count in millions per cu mm | 7.54 ±0.23 | 6.56 ±0.21 | 88 |
| Hematocrit in percent | 42.3 ±0.63 | 33.2 ±1.24 | 79 |
| Hemoglobin in g per 100 ml | 14.7 ±0.26 | 11.3 ±0.21 | 77 |
| Oxygen consumption in liters per square meter body surface per hour | 8.19 ±0.36 | 5.28 ±0.44 | 65 |

Tables III and IV show that the so-called erythropoietic factor and growth hormone, both of which, in our hands, do not correct post-hypoph-

TABLE III

The Effects of "Erythropoietic Factor" on Oxygen Consumption in Adult Female Hypophysectomized Rats. Injections Were Started the Day Following Hypophysectomy—50 μg per Day for 30 Days Followed by 100 μg per Day for 20 Days. ± = Standard Error

| | Normal controls | Hypophysectomized no treatment | | Hypophysectomized plus erythropietic factor | |
|---|---|---|---|---|---|
| | 8 Rats | 5 Rats | % of Normal | 5 Rats | % of Normal |
| Oxygen consumption in liters per square meter body surface per hour | 7.97 ±0.26 | 5.97 ±0.24 | 75 | 5.98 ±0.22 | 75 |

TABLE IV

Effects of Hypophysectomy Alone or Hypophysectomy for 75 Days Followed by Growth Hormone for 50 Days on Oxygen Consumption of Adult Female Rats of the Sprague-Dawley Strain. ± = Standard Error

| | Normal controls | Hypophysectomized no treatment | | Hypophysectomized plus growth hormone | |
|---|---|---|---|---|---|
| | 28 Rats | 22 Rats | % of Normal | 15 Rats | % of Normal |
| Oxygen consumption in liters per square meter body surface per hour | 9.27 ±0.19 | 7.49 ±0.39 | 81 | 6.43 ±0.20 | 69 |

TABLE V

The Blood Picture and Oxygen Consumption of Adult Female Rats 100 Days after Hypophysectomy and after Treatment with 0.005 mg of Thyroxin + 0.6 mg of Cortisone per Day. Treatment Started 50 Days after Hypophysectomy and Continued for 50 Days. ± = Standard Error

| | Normal controls | Hypophysectomized no treatment | | Hypophysectomized thyroxine + cortisone | |
|---|---|---|---|---|---|
| | 15 Rats | 11 Rats | % of Normal | 8 Rats | % of Normal |
| Erythrocyte count in millions per cu mm | 8.31 ±0.08 | 7.81 ±0.17 | 94 | 8.44 ±0.14 | 102 |
| Hematocrit in per cent | 44.3 ±0.58 | 36.6 ±0.77 | 83 | 46.2 ±0.74 | 104 |
| Hemoglobin in g per 100 ml | 14.7 ±0.55 | 12.6 ±0.24 | 86 | 15.5 ±0.25 | 105 |
| Oxygen consumption in liters per square meter body surface per hour | 8.77 ±0.22 | 6.36 ±0.20 | 73 | 8.44 ±0.72 | 96 |

ysectomy anemia, have no effect upon oxygen consumption. Table V shows that combined thyroxin-cortisone therapy induces normal oxygen consumption and a simultaneous normal blood picture in hypophysectomized rats. This is as far as we have progressed in testing the various therapies. At this time, however, the results are not contrary to an "oxygen need" theory.

Most of the above is indirect evidence. We would like to provide direct evidence by injecting some substance into our hypophysectomized rats which will induce an increase in oxygen consumption without simultaneously stimulating general metabolism. Dinitrophenol is such a substance and is quite effective in acute experiments on normal animals, but quite toxic when given over long periods of time to the rather delicate hypophysectomized animal; our experiments with this substance were failures in that small doses were ineffective and larger doses lethal.

## SUMMARY

The hypophysis has a profound influence on erythropoiesis which we believe to be secondary rather than primary. Post-hypophysectomy anemia, in our opinion, develops because of the loss of thyrotropic and adrenocorticotropic hormones and possibly of growth hormone. A theory has been presented that the loss of these hormones finally results in a decreased need for oxygen by the tissues which, in turn, causes a decrease in the rate of erythropoiesis in these animals.


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# Influence of Humoral Factors on Erythropoiesis

*By* ALBERT S. GORDON, PH.D.*

THE PROCESSES of erythropoiesis constitute a complex chain of events which can proceed only through the active participation of many interjoined chemical and physiologic links. Implicated in the fabrication of the red blood cells are the organic substrates—protein, fat, and carbohydrate or their derivatives,[1-3] inorganic elements, principally iron and copper,[2,4,5] and accessory factors including various members of the vitamin family, especially the cobalamines and folic acid.[2,6,7] Each of these factors is indispensable for normal erythropoiesis and total exclusion of any from the dietary regimen is followed by some qualitative, as well as quantitative, aberration in this process.

Serving essentially a facultative rather than an obligatory role in normal erythropoiesis are the hormonal principles. Evidence has accumulated to show that these act more likely by altering the rate rather than by exercising an absolute control over the blood-building processes. It is now well documented that endocrine deprivation, achieved through hypophysectomy, thyroidectomy or adrenalectomy, results in the development of an anemic state, correctable by the administration of the particular hormones that are lacking.[8,11]

Illustrative of this is the anemia which develops after pituitary removal. Within two months following hypophysectomy, a reduction of approximately 25 per cent in red cell count occurs accompanied by decreases in hematocrit and hemoglobin levels.[12] The anemia cannot be attributed to the reduced food intake characteristic of the post-hypophysectomy state or to the trauma incident to the operation.[9] It is slightly microcytic and hypochromic in nature.[12] Use of the red-cell dilution method with $P^{32}$ labeled erythrocytes by Berlin and his associates[13] indicates that a 45 per cent reduction in total circulating red-cell volume follows pituitary removal in the rat. The bone marrow shows hypoplasia associated with marked infiltration of fat and suffers an approximately 40 per cent reduction in total cellularity four months following the operation.[14] A significant reduction in the erythroid-myeloid cell ratio also occurs.[12]

Among the substances found to be most effective in augmenting erythropoiesis in the endocrine-deficient rat are testosterone, thyroxine, adrenal cortical steroids[9,10] and the growth factor.[15,16] Combinations of hormones are more effective than single principles. Thus Crafts and Meineke[17] have demonstrated the potent erythropoietic stimulating qualities of the combination of cortisone and thyroxine, whereas our own studies[10] have indicated the ability of a mixture of growth hormone, testosterone and thyroxine to restore the peripheral as well as the marrow erythrocytic picture to normal levels in hypophysectomized rats.

Thus there can be little argument with the contention that hormones exert significant effects upon erythropoietic processes. It has been proposed by some, moreover, that the endocrine system is essential for the erythropoietic response to normally operating stimuli such as hypoxia. In this connection, Meyer

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and his co-workers[18] demonstrated that the hypophysectomized rat fails to respond with increased erythropoiesis to the stimulus of lowered barometric pressures corresponding to 411 mm Hg(16,000 ft), a pressure of sufficient intensity to stimulate red-cell formation in the normal rat. We decided to repeat these experiments for we felt that the employment of this single intensity of pressure did not explore sufficiently the potential erythropoietic capacities of the hypophysectomized rat. We were able to confirm the results of Meyer and his group.[19] However, upon employing lower

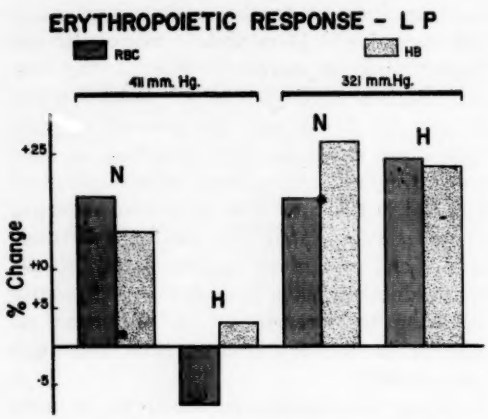


Fig. 1. Red cell and hemoglobin responses in 14 intact (N) and 14 hypophysectomized (H) rats (two months postoperatively) exposed for seven days to two different intensities of reduced barometric pressures. From: Gordon, A. S., *Recent Progress in Hormone Research* 10: 339, 1954, Academic Press, N. Y.

pressures corresponding to 321 mm Hg(22,000 ft) it was possible to demonstrate that the hypophysectomized rat does respond with elevated red-cell counts, hemoglobin values (Fig. 1) and bone marrow hyperplasia to an extent approximately similar to that experienced by intact controls exposed to the same stimulus. It would thus appear that the pituitary is not obligatory for the erythropoietic response to hypoxia and much the same situation prevails for other endocrine organs including the thyroid,[20] gonads[21] and adrenal.[22]

These findings, however, do not preclude the possibility that other humoral mechanisms, apart from the orthodox endocrine system, operate in the erythropoietic response to hypoxia. Since 1906 when Carnot and Déflandre[23] proposed the concept of a circulating hemopoietic factor, termed "hémopoietine," attempts have been made to demonstrate erythropoietic stimulating activity in the body fluids of animals exposed to hypoxic stimuli. The reader is referred to several references on this subject.[24–27]

An account will be given of some of our more recent contributions to this field. Some phases of this work have been done in collaboration with Drs. S. J. Piliero, P. T. Medici, A. L. Luhby and B. Pansky and the Messrs. C. D. Siegel, M. Tannenbaum, J. W. Winkert, J. J. Rolnick and P. Arnow.

### ERYTHROPOIETIC EFFECTS OF PLASMA OBTAINED FROM SINGLY BLED RATS

In this series of experiments, attempts were made to determine whether enhanced erythropoietic activity would appear early in the blood following application of a hypoxic stimulus. Sixty adult male rats of a modified Long-Evans strain were bled an amount equivalent to one-third of their blood volume. Twenty-four hours later, at which time the hematocrits of these animals were reduced 30–40 per cent, they were exsanguinated and the plasma, in 3 ml quantities, was injected intraperitoneally daily for six days into nine intact female rats of the same strain. Plasma obtained from blood drawn from these animals on the first day constituted the control material and was administered in the same way to another group of nine rats.

Significant increases in red cell numbers and reticulocytes occurred in the rats receiving the plasma from the anemic animals. Hemoglobin values showed a slight increase but hematocrit values were unaffected, thus characterizing the response as microcytic. More substantial evidence of the erythropoietic stimulating action of the plasma is provided in the femoral bone marrows of the recipient rats where significant gains in the concentrations of nucleated red cells resulted (Fig. 2). The numbers of immature neutrophilic forms were also elevated but none of the other

marrows values was affected significantly by this treatment. Control plasma displayed no erythropoietic stimulating activity as shown from its lack of ability to influence any of the peripheral or marrow erythrocytic parameters.

One would expect, on *a priori* grounds, that if a humoral mechanism were involved in erythropoiesis, it should start its operation before any distinct morphologic change becomes manifest in the blood-forming organs. The experiments just cited indicate that significant erythropoietic stimulatory activity is detectable in the blood of rats within 24 hours after a single hemorrhage, which corresponds to a time at which as yet no significant elevations in the peripheral reticulocyte levels can be observed. It would be important to know whether "erythropoietin" makes its appearance even before this time.

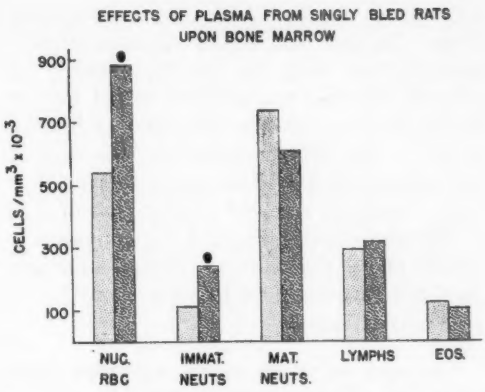


Fig. 2. Mean cellular concentrations in femoral bone marrows of rats receiving daily injections for six days of 3 ml plasma from normal rats (first of each pair of bars) and from these same rats at 24 hours after a single bleeding (second of each pair of bars). Dark ellipse over bar indicates a significant difference ($P > 0.05$) between means. (Rolnick and Gordon, unpublished.)

### ERYTHROPOIETIC STIMULATING EFFECTS OF WHOLE SERUM AND SERUM EXTRACTS FROM RATS MADE ANEMIC WITH PHENYLHYDRAZINE

One of the more effective ways of evoking the production of erythropoietic stimulating factors in the blood is by inducing an anemia with hemolytic agents such as phenylhydrazine. In the present experiments, 100 rats were rendered severely anemic by six daily subcutaneous injections of 5 mg phenylhydrazine hydrochloride. No treatment was administered on the seventh day. On the eighth day the rats were exsanguinated and 4 ml of serum obtained from the blood of these animals were injected daily subcutaneously for 10 days into 12 intact adult female rats of the same strain. Tests for phenylhydrazine in the serum, as determined by the osazone reaction, were negative. Serum secured from 40 normal rats constituted the control material and was administered in a similar manner to five adult female rats.

It will be noted from Figure 3 that the serum from the anemic rats induced significant increases in peripheral red cell, hemoglobin and reticulocyte levels; hematocrit values were unaffected. The "anemic" serum produced an augmentation in the percentages of nucleated red cells in the femoral marrow, thus establishing erythropoietic activity in such sera. None of these effects was apparent in rats receiving injections of whole serum obtained from the normal unbled rats.

Previous experiments[28–34] have indicated that the erythropoietic factor obtained under these conditions is heat-stable. It seemed important to compare the effects of the whole

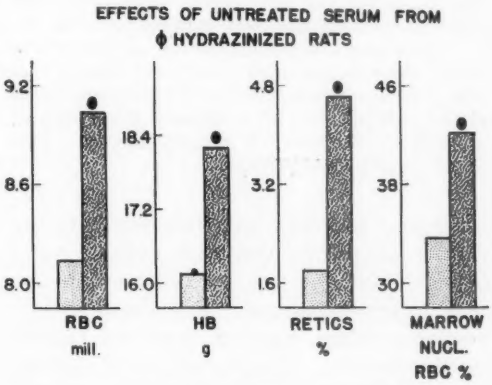


Fig. 3. Mean red cell (RBC), hemoglobin (HB), reticulocyte (RETICS) and marrow nucleated red cell values after daily injections for ten days of 4 ml serum from normal rats (first of each pair of bars) and from phenylhydrazinized rats (second of each pair of bars). Dark ellipse over bar indicates a significant difference ($P < 0.05$) between means. (P. T. Medici, unpublished)

serum with the boiled filtrates derived therefrom. To this end, serum obtained from 40 rats injected daily for six days with 5 mg phenylhydrazine was acidified to pH 5.5 and boiled for 15 minutes as suggested by Borsook *et al.*[35] The filtrate collected was injected subcutaneously for 10 days into intact female rats. Each rat received a daily dose of 4 ml of filtrate (adjusted to pH 8.0), equivalent to 3.2 ml of the original whole serum. The osazone test revealed no detectable phenylhydrazine in the filtrate.

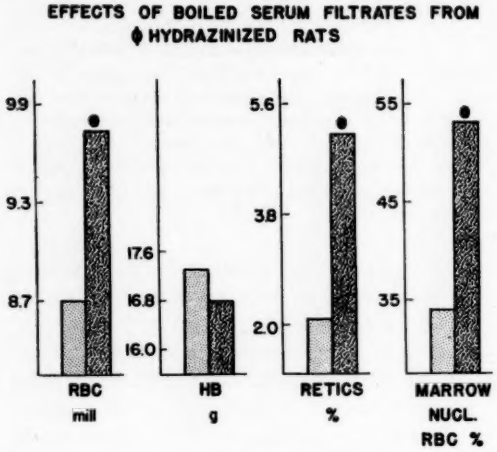


Fig. 4. Mean red cell (RBC), hemoglobin (HB), reticulocyte (RETICS) and marrow nucleated red cell values after daily injections for ten days of 4 ml boiled serum filtrate from normal rats (first of each pair of bars) and from phenylhydrazinized rats (second of each pair of bars). Dark ellipse over bar indicates a significant difference ($P < 0.05$) between means. (P. T. Medici, unpublished.)

Figure 4 indicates that treatment with this extract, as with the untreated serum, resulted in significant gains in red cell numbers and reticulocyte percentages. Hematocrit and hemoglobin values, however, were not affected by the extract. An especially good response was observed in the erythroid cell population of the marrow. Here the increase in percentages of nucleated red cells was even greater than that observed with the whole untreated serum.

The results show that the serum obtained from rats rendered severely anemic by phenylhydrazine significantly stimulates erythropoiesis in normal rats of the same strain. It is apparent also that the factor is heat-resistant and is recoverable, in full activity, in boiled filtrates of the serum. In fact, stimulation of erythropoiesis in the marrow appears greater with the filtrate than with the whole serum.

The failure to obtain significant rises in hematocrit values with either the whole serum or the boiled filtrates of sera is in accord with the observations of Linman and Bethell[34] and ourselves[36] that microcytosis sometimes characterizes the response to the erythropoietic factor. This may imply that, under the particular conditions of the experiment, attainment of a normal red cell size has not kept pace with the increased rate of cellular division.

### ATTEMPTS TO IDENTIFY THE SITE OF PRODUCTION OF THE ERYTHROPOIETIC FACTOR*

Relatively few investigations have dealt with the site of formation of the erythropoietic principle.[36] It seemed important to consider this problem in greater detail by subjecting a variety of organs to the chemical procedures employed by us and others for revealing erythropoietic activity in plasma.

In these experiments, eight New Zealand rabbits weighing 4 to 6 kg were injected daily for two days with 1 ml of a 2.5 per cent solution of phenylhydrazine hydrochloride (25 mg) neutralized to pH 7.1. On the third day, and for the subsequent two days, they received 1.5 to 2.5 ml of this solution (37.5 to 62.5 mg) graded according to body weight. No treatment was administered on the sixth day because of the death of two animals. The remaining six animals were intensely anemic on the seventh day, displaying red cell counts of approximately 1 million/cu mm and reticulocyte values ranging from 82 to 96 per cent. At this time, the pooled plasmas of these animals were acidified to pH 5.5, boiled for ten minutes, filtered, and the filtrates collected. The livers, spleens, thymuses, lungs, brains (excluding medullae and pituitaries), gastrocnemii, bone marrows (femoral, tibial and humeral) and packed blood cells (82 to 96 per

* Taken from Gordon *et al.*[36]

cent reticulocytes) were collected and the respective organs pooled. All organs were homogenized in 100 to 400 ml of acid saline for 20 minutes in a Waring blendor. The homogenates were acidified further to pH 5.5 and boiled for 10 minutes, after which the filtrates were collected.

Nine groups, each consisting of five normal adult female rats, were employed to assess the hemopoietic activities of the nine filtrates. They were given daily subcutaneous injections, for ten days, of the following amounts of extract: plasma—1 ml, equivalent to 2.9 ml of original whole plasma; liver—3 ml, equivalent to 6 g tissue; spleen—1.5 ml, equivalent to 0.39 g tissue for the first five days followed by 5 daily 2 ml injections; thymus—1.2 ml, equivalent to 0.6 g tissue; lung—2 ml, equivalent to 1.4 g tissue; brain—1.3 ml, equivalent to 1.04 g tissue; muscle—3 ml, equivalent to 5.7 g tissue; bone marrow—3 ml, equivalent to 1 g tissue and packed cells—2 ml, equivalent to 3 ml of cells.

The results indicated that, of all the filtrates tested, only that obtained from plasma stimulated erythropoiesis in the recipient rats (Fig. 5). Thus red cell numbers were increased 30 per cent, hematocrits 16 per cent, reticulocytes 100 per cent, nucleated red cell percentages 48 per cent. A trend towards an increase in hemoglobin of 10 per cent was also apparent with the plasma extract.

The absence of erythropoietic stimulating activity in the organ extracts employed in this study need not necessarily be interpreted as meaning that these organs do not participate in the production of the erythropoietic factor. It is conceivable that an efficient storage mechanism for the principle does not exist, so that the factor is released into the blood as rapidly as it is produced. The present results indicate, therefore, that plasma may be the only site from which appreciable amounts of the erythropoietic factor may be recovered.

Recent experiments performed in our laboratory have indicated the possibility that certain enzymatic patterns of bone marrow can be used as indicators of erythropoietic stimulation. Thus single or multiple bleedings, inorganic cobalt or phenylhydrazine result in a significant lowering of the diglycinase, while exerting smaller and, in some instances, no consistent changes in the triglycinase activity of bone marrow. The activity of these peptidases was determined by the micro-alkali titration method of Grassmann and Heyde.[37]

Table I indicates that boiled plasma filtrates obtained from rabbits treated with phenylhydrazine also lowered the diglycinase activity of the marrows of the recipient rats. This

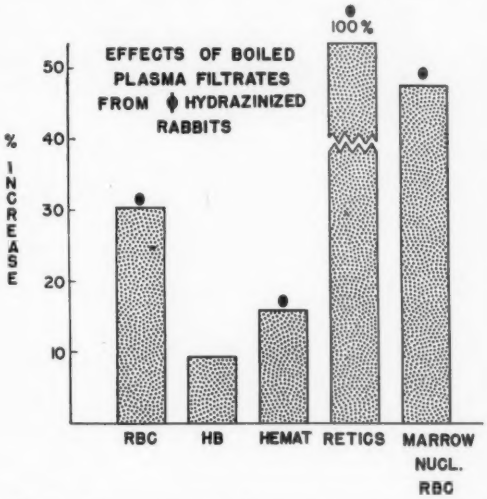


Fig. 5. Mean percentage increases in red cell (RBC in millions/cu mm), hemoglobin (HB in g/100 ml), hematocrit (HEMAT in per cent), reticulocyte (RETICS in per cent) and marrow nucleated red cell (per cent) values in rats following 10 daily injections of 1 ml boiled plasma filtrate from phenylhydrazinized rabbits. Dark ellipse over bar indicates significant increases ($P < 0.05$) compared to control values. (Taken from Gordon *et al.*[36])

was true whether the activity was expressed on a nitrogen or cellular number basis. There was a tendency for this enzyme activity to decrease after treatment with the spleen and packed red cell extracts, but these decreases were just within the limits of significance and may represent the effects of small amounts of residual blood in these filtrates. No definable changes in the pattern of triglycinase activity were discerned with any of the extracts employed. Thus erythropoietic stimulating activity appears to be associated with a decrease in the dipeptidase activity of

TABLE I

Influence of Acidified Boiled Extracts of Plasma and Tissues from Phenylhydrazinized Rabbits upon Dipeptidae and Tripeptidase Activity of Rat Bone Marrow§

| Extract employed | | Glycylglycinase activity | | Glycylglycylglycinase activity | |
|---|---|---|---|---|---|
| | | Cell no. basis† | C (X $10^4$)‡ | Cell no. basis† | C (X $10^4$)‡ |
| None | (6)* | 6.8 ± 0.5 | 64.1 ± 5.5 | 29.8 ± 1.8 | 196 ± 4.7 |
| Plasma | (5) | 3.1 ± 0.2 | 30.2 ± 1.2 | 23.4 ± 2.8 | 214 ± 4.6 |
| Liver | (5) | 6.8 ± 0.9 | 60.0 ± 3.9 | 27.0 ± 0.9 | 229 ± 5.8 |
| Spleen | (4) | 4.1 ± 0.8 | 52.0 ± 4.1 | 22.5 ± 1.1 | 200 ± 5.1 |
| Packed RBC | (4) | 4.8 ± 0.6 | 45.0 ± 2.5 | 25.7 ± 0.9 | 215 ± 4.5 |
| Bone marrow | (3) | 6.9 ± 0.6 | 59.0 ± 4.9 | 29.8 ± 1.7 | 221 ± 8.4 |
| Thymus | (4) | 7.0 ± 0.7 | 57.8 ± 2.6 | 25.1 ± 1.3 | 211 ± 6.1 |
| Brain | (3) | 7.2 ± 0.5 | 57.0 ± 3.7 | 27.8 ± 1.2 | 209 ± 5.6 |
| Lung | (5) | 6.9 ± 0.4 | 59.1 ± 4.8 | 26.8 ± 0.5 | 219 ± 5.9 |
| Muscle | (4) | 6.7 ± 0.5 | 61.0 ± 4.1 | 28.2 ± 1.3 | 213 ± 8.1 |

* Number of rats.

† Represents per cent hydrolysis of substrate per $10^7$ nucleated marrow cells per 2 hours (diglycinase) or per hour (triglycinase). Figures are given as Means ± Standard Errors.

‡ C, the proteolytic coefficient, was calculated from $K_1/E$ where $K_1$, the first order rate constant, was determined from the equation—$K_1$ equals log (100/100 minus the per cent hydrolysis)/t (minutes) and E represents the protein nitrogen in mg per ml of the final test solution.[38]

§ Tannenbaum and Gordon, unpublished.

bone marrow. The precise significance of this finding remains to be clarified but it points to the possible utilization of this parameter in assessing the relative activities of erythropoietic agents.

### ERYTHROPOIETIC-STIMULATING EFFECTS OF PLASMA EXTRACTS FROM PATIENTS WITH COOLEY'S ANEMIA*

The repeated demonstration of erythropoietic-stimulating activity in the blood of animals exposed to anoxic conditions spurred our attention to the question as to whether such effects are also demonstrable in the blood of patients possessing various forms of anemia. Since the animal experiments appear to indicate that more erythropoietic activity is recoverable in the hemolytic than in other types of experimentally induced anemia, probably because the hemolytic varieties are usually more severe, it was felt that an examination of the effects of blood secured from patients with Cooley's anemia might be revealing.

Plasma was obtained from seven authenticated cases of Cooley's anemia (six children and one adult) and one case of chronic hypoplastic anemia (erythroid hypoplasia). Four normal children served as donors of control plasma.

Because of the effectiveness of the acidification-boiling procedure in exposing erythropoietic activity in the blood of anoxic rodents, it was decided to utilize this extraction method for the plasmas of the anemic patients. The plasmas were acidified to pH 5.5 and boiled for 10 minutes. Following filtration, the coagulated residues were discarded and the filtrates neutralized. A similar acidification and boiling procedure was used for a specimen of urine obtained from one of the patients with Cooley's anemia.

Rats were employed to test the erythropoietic stimulating activity of these filtrates. Each animal was injected subcutaneously daily for five days with 1.8 to 3.0 ml of filtrate equivalent in all cases to approximately 3 ml of original plasma. The rats treated with the urine extract received 5 daily injections of 1.5 ml urine extract, each equivalent to 3 ml of the original urine. All rats were killed on the sixth day for erythrocytic studies of the peripheral blood and bone marrow.

The effects of the plasma filtrates for two representative cases of Cooley's anemia are

* Taken from Piliero *et al.*[39]

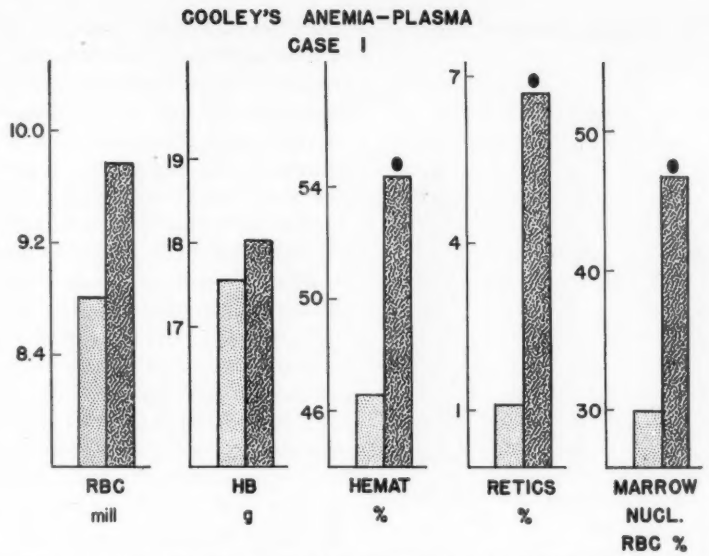


Fig. 6. Mean red cell, hemoglobin, hematocrit, reticulocyte and marrow nucleated red cell values in rats before (first of each pair of bars) and after (second of each pair of bars) five daily injections of plasma filtrate from a 5 year old boy with Cooley's anemia (hemoglobin—5.5 g/100 ml). Dark ellipse over bar represents a significant difference ($P < 0.05$) between means. (Taken from Piliero *et al.*[39])

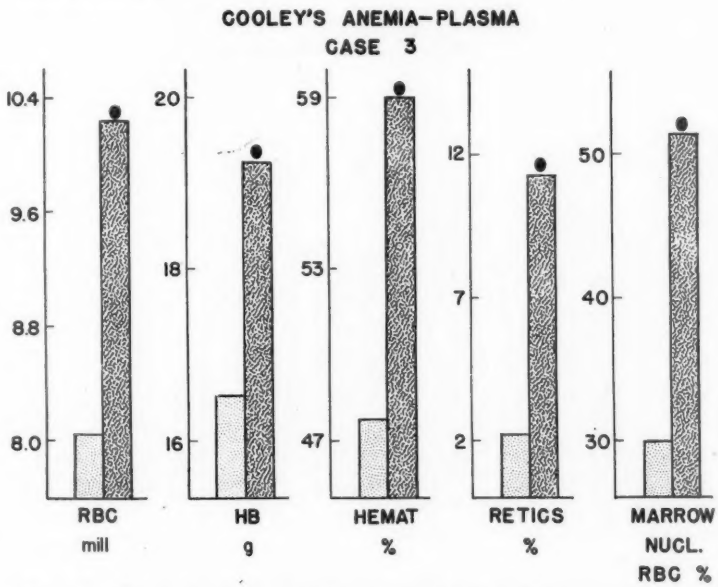


Fig. 7. Mean red cell, hemoglobin, hematocrit, reticulocyte and marrow nucleated red cell values in rats before (first of each pair of bars) and after (second of each pair of bars) five daily injections of plasma filtrate from a 5 year old girl with Cooley's anemia (hemoglobin—5.0 g/100 ml). Dark ellipse over bar represents a significant difference ($P < 0.05$) between means. (Taken from Piliero *et al.*[39])

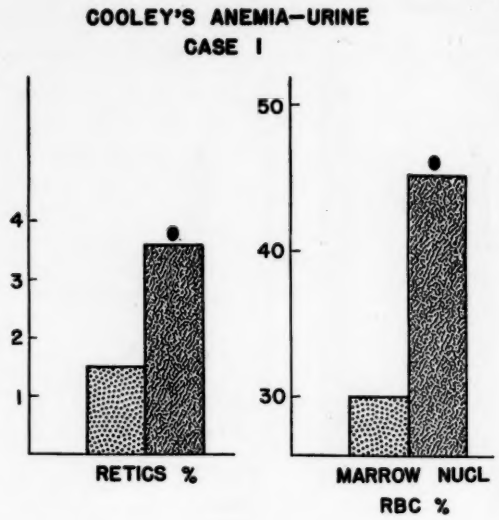


Fig. 8. Mean reticulocyte and marrow nucleated red cell values in rats before (first of each pair of bars) and after (second of each pair of bars) five daily injections of urine extract from the boy with Cooley's anemia shown in Figure 6. Dark ellipse over bar represents a significant difference ($P < 0.05$) between means. (Taken from Peliero *et al.*[38])

depicted in Figures 6 and 7. It will be observed that in both instances, significant increases in hematocrit, reticulocyte and marrow nucleated red cell values occurred. In Case 1 (Fig. 6), a trend toward a rise in red cell numbers and hemoglobin concentrations was observed, whereas in Case 2 (Fig. 7), the increases in these values were more distinct.

The extract of urine obtained from the child with Cooley's anemia (Case 1) also stimulated erythropoiesis, as judged by the significant reticulocytosis and the increased percentages of nucleated red cells in the marrows of the recipient rats (Fig. 8).

Chronic hypoplastic anemia was present in a boy since birth. Sternal biopsy indicated less than 2 per cent nucleated red cells in his bone marrow and his red cell count, at the time of obtaining the blood sample, was 1.8 million/cu mm. Despite the intensity of the anemia in this subject, no erythropoietic stimulating activity was demonstrable in the extract prepared from his plasma (Fig. 9).

None of the extracts obtained from the control donor subjects stimulated erythro-

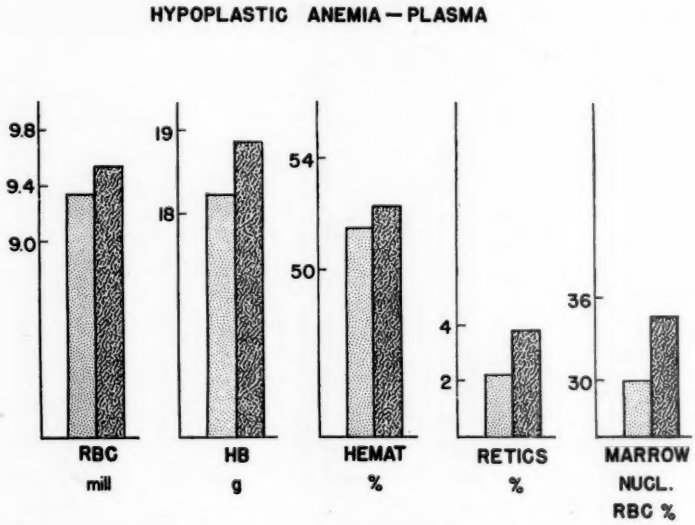


Fig. 9. Mean red cell, hemoglobin, hematocrit, reticulocyte and marrow nucleated red cell values in rats before (first of each pair of bars) and after (second of each pair of bars) five daily injections of plasma filtrate from a 7 year old boy with chronic hypoplastic anemia (hemoglobin5. 0 g/100 ml). None of the differences between the means is significant. (Taken from Piliero *et al.*[38])

poiesis in the recipient rats. In fact, distinct decreases in the hemoglobin and hematocrit values were noted with two of these extracts (one case is illustrated in Figure 10).

The results thus indicate that considerable erythropoietic stimulating activity exists in the blood of patients with Cooley's anemia. The erythropoietic factor detected here is heat-stable, being recoverable in boiled filtrates of the plasma. In these respects, it resembles the material present in the plasma of rabbits and rats subjected to hemolytic or hemorrhagic anemia (vide supra). The activity of human circulating "erythropoietin," as seen in some of the patients, e.g., Case 3, is actually greater than that detectable in animals treated with phenylhydrazine or subjected to bleedings, considering that the material of human origin was administered for only five days. This may mean that the human circulating factor is more portent than that of animal origin or, what is more likely, the factor exists in larger concentration in the blood of the anemic patient studied.

It was of interest that the urine extract of one of the subjects with Cooley's anemia possessed erythropoietic stimulating qualities. Considering that the urine was concentrated to no more than 50 per cent of its original volume, the reticulocyte and marrow response in the recipient rats represents a considerable degree of erythropoietic activity. Others[26] have also detected such activity in boiled extracts of urine from rabbits subjected to bleedings.

A significant finding was the absence of erythropoietic activity in the plasma extract of the patient with chronic hypoplastic anemia. It is possible that there is an impaired capacity of such subjects to elaborate the erythropoietic principle. Should this prove to be the case in more detailed studies of hypoplastic anemia, the administration to these subjects of purified "erythropoietin" would constitute a justifiable therapeutic measure.

### CHEMICAL NATURE OF THE CIRCULATING ERYTHROPOIETIC FACTOR

The action of the circulating erythropoietic principle cannot be attributed to extra quantities of known blood-stimulating substances such as vitamin $B_{12}$ or folic acid in the extracts.[40] The material in the blood of rabbits subjected to repeated bleedings or phenylhydrazine treatment is said to be heat stable and soluble in alcohol, ether and acetone.[28,41] Activity is also displayed by acetone or water

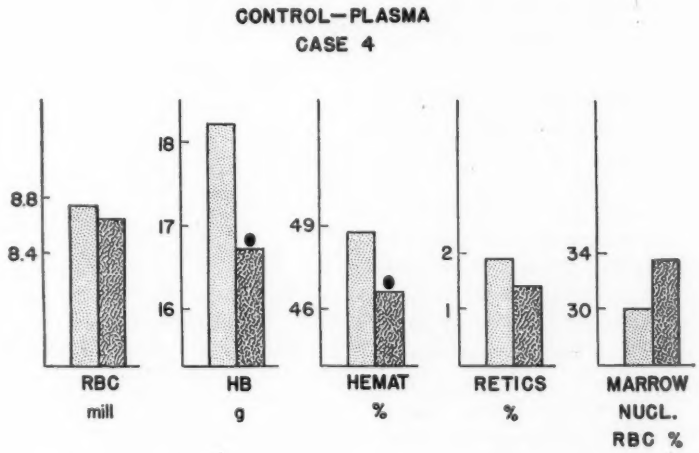


Fig. 10. Mean red cell, hemoglobin, hematocrit, reticulocyte and marrow nucleated red cell values in rats before (first of each pair of bars) and after (second of each pair of bars) five daily injections of plasma filtrate from a normal 6 year old boy. Dark ellipse over bar represents a significant difference ($P < 0.05$) between means. (Taken from Piliero *et al.*[39])

extracts of the heat-coagulated proteins of plasma from bled rabbits.[42] The material obtained under such conditions is claimed to be dialyzable through cellophane and experiences reduced activity when exposed to oxygen.[42] Chromatographic analysis of the purified acetone extracts[42] shows a "positive color reaction with Sudan IV (fatty acid esters) and negative reactions with ninhydrin (amino acids), mercuric nitrate reagent (purines) and ammoniacal silver nitrate (sugars)." Gley and Delor[43] have hypothesized two separable erythropoietic factors in the blood of animals subjected to hemorrhage, one lipoid-soluble called "hématopoïetine," and the other water-soluble named "hématostimuline." The action of the latter is said to be indirect. Organic extraction procedures coupled with infrared spectrophotometry suggest to these authors[43] that the primary factor "hématopoïetine" is a steroid possessing one functional alcoholic and three functional ketonic groups. The experiments of Linman and Bethell[34] indicate that the activity in boiled filtrates of plasma obtained from bled or phenylhydrazinized rabbits is not precipitated by perchloric acid and that it may be ether-soluble. Other studies[42,44] show that the factor is precipitable from whole serum or plasma with ammonium sulphate and is apparently associated with the albumin and alpha- and beta-globulin fractions.

Our own studies on the characterization of the factor have indicated that the erythropoietic stimulating activity cannot be extracted with petroleum ether or with ethyl acetate from boiled plasma filtrates from phenylhydrazinized rabbits. Chromatographic technics reveal no specific qualitative differences between the active and control filtrates of rabbit plasma with respect to the following substances: free amino acids (phenol-water, butanol-acetic acid-water; ninhydrin), purines and pyrimidines (butanol-HCl; ultraviolet light), sugars (butanol-acetic acid-water; ammoniacal silver nitrate) as well as organic acids (ether-acetic acid-water; ammoniacal silver nitrate). Likewise, preliminary runs performed on the plasma filtrates from patients with Cooley's anemia and control subjects show no consistent differences in the free amino acid patterns.

We have found recently that when the cationic dye, *toluidine blue O* is added to the boiled plasma filtrates, metachromasia in the chromatograms develops with those filtrates which possess erythropoietic stimulating activity. The intensity of the metachromatic reaction appears fairly well correlated to the degree of biological activity. The data are indicated in Table II.

TABLE II

Correlation of Metachromasia and Erythropoietic stimulating Activity in Plasma Filtrates from Rabbits and Human Subjects†

| Subjects with Cooley's anemia | Increase in reticulocytes in recipient rats (numbers per 100 RBC) | Degree of metachromasia |
|---|---|---|
| 1 | 9.1 | 2+ |
| 2 | 5.6 | 4+ |
| 3 | 3.7 | 2+ |
| 4 | 0.2* | 2+ |
| 5 | 0.9 | 0 |
| 6 | 0.1 | 0 |
| Control human subjects: | | |
| 1 | 0.3 | 0 |
| 2 | 0.8 | 0 |
| Phenylhydrazinized rabbits: | | |
| 1 | 3.1 | 3+ |
| 2 | 1.4 | 1+ |
| Normal rabbit: | | |
| 1 | 0.6 | 0 |

* Despite the insignificant reticulocyte response, a significant increase in the numbers of marrow nucleated red cells occurred in the rats receiving this extract.

† Gordon, Piliero, Medici, Luhby, Winkert and Arnow, unpublished.

The precise significance of the metachromatic reaction in the active plasma filtrates remains to be determined. Rambach[45] has stated that the erythropoietically active material seems to be a mucoprotein associated with the alpha$_2$-globulin fraction and Borsook[46] also agrees that it is a mucoprotein. Since mucoproteins in general are known to resist the acidification and boiling procedure used in the preparation of our active extracts, the erythropoietic stimulating factor may be a

mucoprotein or a substance similar to, or associated with it.


## SUMMARY

It has been shown that although the endocrine glands, including the anterior pituitary, thyroid and adrenal, exert significant effects upon erythropoiesis, their role is a subsidiary one. They operate probably by altering the rate rather than by exerting an absolute control over the erythropoietic processes.

There can be no doubt that erythropoietic stimulating activity appears in the blood of animals and man under anoxic conditions. The factor(s) evoked by anoxia is heat-stable and appears in greater concentration in the plasma than in a variety of organs examined. Its presence in large concentration in the blood of human subjects with Cooley's anemia and its absence in a case of chronic hypoplastic anemia suggest that it plays a part in erythropoiesis in man and presents the possibility of its utilization in the treatment of certain types of anemia.

Opinion is divided concerning the chemical nature of the erythropoietic factor. A lipoidal substance (e.g., fatty-acid ester or steroid) is suggested by some experiments whereas others indicate that it may be protein in nature. Our chromatographic studies show a good positive correlation between the degree of metachromasia and erythropoietic stimulating activity in boiled filtrates of plasma obtained from subjects with Cooley's anemia, rabbits treated with phenylhydrazine and from control subjects. This would support the contention that the erythropoietic factor is a mucoprotein or a moiety associated with this fraction.

There is need at the present for more information concerning the site of production of the erythropoietic factor, its chemical nature and mode of action, and its possible application to the treatment of a variety of blood dyscrasias in man and laboratory animals.


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# Nutrition News

### The Nutrition Branch 9th Pacific Science Congress

The 9th Pacific Science Congress will be held in Bangkok, Thailand from November 18 to December 9, 1957 under the auspices of His Majesty's Government of Thailand, and the Science Society of Thailand. Nutritionists, Dietitians, Food Technologists, and other persons interested in or doing work on food, diet, nutrition, etc., are invited to participate in the Nutrition Branch of the Congress. The technical meetings, discussions, and symposia will take place during the first two weeks. The third week will be devoted to scientific and technical excursion. Details and other relevant documents may be obtained from the Secretariat, Department of Science, Ministry of Industry, Bangkok, Thailand.

### Diabetes Program Guide

A Diabetes Program Guide, written specifically to provide information to communities interested in engaging in diabetes program activities, is now available. Included therein are the results of original investigation by the Chronic Disease Program of the U. S. Public Health Service, such as case-finding, validity and interpretation of tests, and blood preservation.

Copies of this Guide may be purchased from the Superintendent of Documents, Government Printing Office, Washington 25, D. C., at 45 cents per copy.

### Lipotropic Research Foundation Grants-in-aid

The Lipotropic Research Foundation of New York will receive applications for grants-in-aid for 1958 until August 15, 1957, with special attention being given to clinical studies. A request for application forms, which should include a short statement describing professional affiliations and experience, may be addressed to the administrative secretary, Dr. L. Lipton, 26 Vark Street, Yonkers 1, New York.

## Coming

The September–October 1957 issue of THE AMERICAN JOURNAL OF CLINICAL NUTRITION will be of unusual interest. The entire issue will be devoted to a Symposium on the Interrelationship Between Pediatric Disorders and Nutrition under the guest editorship of Paul György, M.D., and L. Emmett Holt, Jr., M.D. Some of the topics to be covered are: Failure to Gain Weight; Clinical and Biochemical Observations in Galactosemia; Iron and Copper in Infant Nutrition; The Pathogenesis of Interrelationship Between Pediatric Disorders and Nutrition; The Evaluation of Artificial Feeding.

# Nutrition Briefs

MONGOLOID children show a decrease in Vitamin A absorption. Their serum calcium and albumin are lower while the gamma globulin are higher than normal children of the same age. There is a possibility that the initial cause of mongolism may be related to an inadequate transfer of vitamin A from mother to fetus.
—A. E. Sobel, *et al.* *Clin. Chem.* 2: 289, 1956.

USING inbred mice, it was found that the life span and cause of death could be affected by dietary changes. A metabolic disorder (heart failure syndrome) could be produced on a special diet and prevented by vitamin E or vitamin $B_{12}$. Convulsive seizures also developed in relation to dietary regimens.
—Y. C. P. Lee, M. B. Visscher and J. T. King. *J. Gerontol.* 11: 364, 1956.

BREAST milk appears to prevent neonatal hypocalcemia. Fresh cow's milk produces a rise in serum phosphorus, while processed milks (evaporated and powdered) produced the highest rise in phosphorus and causes an increased incidence of hypocalcemia during the first week of life.
—J. B. Pincus and I. F. Gittleman. *Clin. Chem.* 2: 290, 1956.

OF SEVEN balance studies on epiphysiolysis patients, six showed subnormal calcium retention and serum phosphorus levels. In four cases in which it was employed, adequate vitamin D was shown to increase calcium retention significantly. Epiphysiolysis is a disease preventable by proper nutritional practice.
—J. A. Johnston, G. Manson, and C. L. Mitchell. *A. M. A. J. Dis. Child.* 92: 337, 1956.

IN A STUDY employing both the rat and rabbit, the administration of iodide was not found to alter the intestinal absorption or excretion of cholesterol. Further, iodide administration was not found to alter either the normal or abnormal disposition of endogenous cholesterol.
—M. Friedman, R. Homer, and S. O. Byers. *J. Clin. Investigation* 35: 1015, 1956.

PERNICIOUS anemia is commoner in persons of blood group A than in persons of group O, and also perhaps commoner than in persons of group B.
—*Brit. M. J.* 2: 723, 1956.

CORTISONE treatment of rabbits resulted in diffuse ductular hyperplasia of the pancreas recognizable after only eight days of treatment and uninfluenced by antibiotics induced by duct ligation. It is thought that cortisone may induce changes in viscosity of pancreatic secretion resulting in obstruction.
—S. A. Bencosme, and S. S. Lazarus. *A. M. A. Arch. Path.* 62: 295, 1956.

AS MUCH AS two-thirds of the small intestine of the rat may be removed without subsequent loss of weight or demonstrable defects of absorption. If more is removed, weight is lost and defective absorption of glucose and iodide by the small intestine can usually be demonstrated.
—P. C. Reynell, and R. Wang. *Gastroenterology* 31: 361, 1956.

PREDNISONE increased the urinary excretion of vitamin $B_{12}$, but decreased the excretion of thiamine and pantothenic acids in rats. The elimination of folic and folinic acids was not changed.
—E. Ginoulhiac and S. Nani. *Acta Vitaminologia* 4: 157, 1956.

THE BLOOD pepsin level (an indirect index of gastric secretion) rises in the seventh month of pregnancy and reaches a peak on the first post partum day, falling to normal in about five months.
—W. A. Gryboski and H. M. Spiro. *N. Eng. J. Med.* 255: 1131, 1956.

GUINEA PIGS injected with alloxan and ascorbic acid simultaneously show a higher fatality rate than those receiving alloxan alone. None developed diabetes. Anemia could not be established with certainty in animals given both drugs.
—N. Allegretti and D. Gabrić. *Arch. Internat. Pharmacodyn.* 107: 353, 1956.

A DIABETIC-like syndrome has been produced in rats by the administration of xanthurenic acid, an abnormal metabolite of tryptophan. A high fat-and-casein and vitamin $B_6$-deficient diet led to a diabetic syndrome with histologic changes in the $\beta$-cells of the islets.
—Y. Kotake. *Clin. Chem.* 2: 262, 1956.

# Reviews of Recent Books

**Essays in Metabolism. The John Punnett Peters Number of the Yale Journal of Biology and Medicine,** edited by Louis G. Welt. Little, Brown, Boston, pp. 382, $6.50.

As a tribute to the late Dr. John P. Peters, a series of ten essays has been prepared by his former associates dealing with those phases of clinical biochemistry and metabolism in which he was pre-eminent for many years. The initial chapter is an appreciation of Dr. Peters by Lavietes providing a clear understanding of the remarkable abilities of the man as a scholar, teacher, and investigator. The original contributions emerge from the investigations currently conducted in the laboratories and clinics of the respective authors. On discussing body-buffer mechanisms in acid-base equilibrium, Elkinton describes the major portion of the physicochemical defense of body fluid neutrality as occurring in phases other than blood involving a series of linked ionic exchanges. The regulation of acid-base balance by renal mechanisms including the carbonic acid system and plasma $CO_2$ tension and ammonia formation is reviewed by Orloff. Sodium and potassium metabolism in experimental animals as affected by the adrenal steroids is presented by Seldin, Welt and Cort. The discussion of water distribution by Rosenbaum should stimulate further investigations into the problem of the *modus operandi* of cellular and extracellular fluid shifts. The regulation of extracellular fluid and sodium content by volume receptors located within the arterial tree which, in turn, influence renal excretory mechanisms is discussed by Epstein.

The excretion of water and solutes as affected by antidiuretic hormone and by intrinsic renal tubular responses to variations in osmotic activity of filtered fluids is presented by Welt. The impairment of water excretion in disease states characterized by the absence of diuresis in response to water load provides the basis for discussion of the renal regulatory mechanisms. The final three essays deal with specific aspects of carbohydrate metabolism including fructose utilization, glucose, electrolyte changes in cirrhosis of the liver, and serum lipid alterations.

The volume contains a wealth of new information expertly integrated with a thorough review of each of the topics discussed. Extensive bibliographies are included covering the important experimental and clinical contributions in the fields of renal physiology, electrolyte and water balance, and carbohydrate metabolism. Clinical investigators and students will find the book of inestimable value in orienting their concepts in these rapidly advancing phases of medicine, while the practising physician may find in its pages a means of coming abreast of current progress in metabolic experimentation.

C. R. SHUMAN

**Experimental Design** by W. T. Federer, The MacMillan Co., New York, 1955, pp. 535, $11.00.

The author of this advanced college textbook is Professor of Biological Statistics, Department of Plant Breeding, N.Y. State College of Agriculture, at Cornell University. The book originated from lecture notes to students and the objectives were to present subject matter and technics not readily available elsewhere. Examples are taken from the field of agriculture and botany, and although the basic principles apply to medical research, nevertheless, this makes for somewhat difficult reading.

As the author states, the reader needs a well-grounded familiarity with statistical concepts and technics before he can appreciate this book. Mathematical theory abounds and a good mastery of calculus and algebra is necessary. Incidentally, in the index no reference to the recently described sequential analysis was found. In short, this book is oriented toward certain non-medical biologic sciences and is quite advanced in the technical sense. It is a scholarly work on an important subject.

S. O. W.

**Liver, Biliary Tract and Pancreas, The Ciba Collection of Medical Illustrations.** Part III of Volume 3, Digestive System by F. H. Netter, Ciba Pharmaceutical Products, Inc., Summit, N. J., 1957, pp. 156, $10.50.

This is an outstanding work. Containing 133 full-color plates for which Frank Netter is justly famous, and including a well-written descriptive text, an excellent bibliography and a most complete index, the magnificent atlas deserves to be seen by every student and graduate physician. In addition to Netter's drawings, and E. Oppenheimer's editing, there were five qualified consultants; all these contributors serve to make the book informative as well as attractive.

Special mention should be made of the section on physiology of the hepatobiliary system. The combination of art and text go a long way in explaining vividly the complexity of liver functions and the tests used to study them. Here is "medical education" in a new form, and one that can be highly recommended.

S. O. W.

**Essentials of Nutrition,** 4th edition by Henry C. Sherman and Caroline Sherman Lanford, The MacMillan Co.. New York, 1957, pp. 505, $4.90.

The fact that this book has reached its fourth edition is in itself testimony to its acceptability as a college text and reference volume.

Both in format and style the material is distinguished for its clarity, readibility and accessibility. The authors presume no scientific training on the part of the reader, and there are no formal definitions. A particularly valuable feature of the book is an excellent 14-page glossary of nutritional and allied terms.

Primarily designed as a college text, the book should prove a welcome addition to the library of anyone desiring a compact little reference volume on the elements of nutrition and the practical application of this knowledge. A. E. S.

**The Physician-Writer's** Book by Richard M. Hewitt, W. B. Saunders, Philadelphia, 1957, pp. 394, $7.00.
**Guide to Medical Writing** by Henry A. Davidson, Ronald Press, New York, 1957, pp. 330, $5.00.

The almost simultaneous appearance of two good books on a neglected subject makes comparison inevitable. Both books are based on extensive editorial experience. Yet the approaches of the two authors differ and each book offers something different to the reader.

Hewitt's book is based on many years of service at the Mayo Clinic and before that with the *J. A. M. A.* It is prepared for the inexperienced author-physician. It is not a style book and not directed primarily to editors. The organization is unusual and, to this reviewer at least, the frequent use of intermingled bold face, large and small roman, and italic types is not entirely appealing. The book considers first the whole article (or book), then follow comments on the paragraph, the sentence, and finally on words. It concludes with 75 pages of appendixes (or appendices according to Davidson) which contain much useful material and should perhaps have better been included in the body of the text.

Davidson's book reads more smoothly, and is in many ways more "practical." That is, it refers specifically to more of the common problems faced by author and editor. I was particularly interested with the manner in which these books handle the problem of trade and generic names of drugs. In this case Davidson gives a full and detailed discussion, whereas Hewitt's comments are brief and scattered. Davidson's book is written with a certain breezy flair and wry humor which is pleasant and unpretentious.

Hewitt's book is full of reminiscences and references to specific individuals which gives it a chatty quality. On the other hand, the large number of these references (which are also found in the index) does not lead to easy reading. The 16-line chapter 18, in this reviewer's opinion could well have been included elsewhere. Nevertheless, Hewitt's book is full of good solid advice and every medical author will profit from it.

Davidson's style is full of unexpected touches, such as "The Zilch Law holds that the less the intellectual content of a spoken paper, the greater the number of lantern slides used. . . . The more the fat the less the meat." The informality at times is overdone, but undoubtedly the message is carried across. This book, too, will teach the reader how to improve his medical writing. The real problem is to make writers follow the valuable advice in these two interesting books. S. O. W.

**Communication: Handling Ideas Effectively** by R. I. Johnson, M. Schalekamp, and L. A. Garrison, McGraw-Hill Book Co., New York, pp. 361, $4.50.

This is a textbook for college courses in "communication" and considers writing, reading, speaking, listening and observing. A wide variety of topics are discussed, among them efficient note taking, making reports, improving pronounciation, developing editorial skills, etc. Since progress in science as in other intellectual activities depends on the effective communication of ideas, the discussions in this book should be of interest to many of our readers. Although some may find it too elementary for their needs, others will appreciate the many psychologic overtones; still others will benefit from the numerous helpful hints on understanding and being understood. R.S.W.

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

*Aktuelle Diabetesfragen* by F. Bertram and J. Kuntze, Georg Thieme Verlag, Stuttgart, 1957, pp. 80, D.M. 5.70.

*Annual Review of Medicine*, edited by David A. Rytand and William Creger, Vol. 8, Annual Reviews, Inc., Palo Alto, California, 1957, pp. 530, $7.00.

*Dietetique du Medecin Practicien*, edited by J. Lederer, Masson et Cie., Paris, 1957, pp. 520, 2,500 fr. paper bound, 3,200 fr. cardboard bound.

*Modern Problems in Pediatrics II* by A. Hottinger and F. Hauser, S. Karger, Basel, Switzerland, 1957, pp. 222, Sw. fr. 32.

*Regulation and Mode of Action of Thyroid Hormones*, edited by G. E. W. Wolstenholme and Elaine C. P. Millar (Ciba Foundation), Little, Brown & Co., Boston, 1957, pp. 311, $8.50.

*The Chemistry and Biology of Purines*, edited by G. E. W. Wolstenholme and Cecilia M. O'Connor (Ciba Foundation), Little, Brown & Company, Boston, 1957, pp. 317, $9.00.

# Abstracts of Current Literature

CHARLES R. SHUMAN, M.D., EDITOR

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## NEOPLASIA AND NUTRITION

*One of the important keys to a better understanding of the behavior of cancer is the knowledge of its nutritional and metabolic requirements. While there is no apparent link between diet and cancer pathogeneses, with isolated exceptions such as carcinoma of esophagus in Scottish women who consume scalding tea, it seems probable that the neoplastic cells would be influenced by nutritional factors. The production of cancer by drinking hot tea or the finding of hepatic carcinoma in cirrhotic livers can scarcely be considered as related directly to the dietary habits. It should however be regarded as a consequence of local irritative or aberrant regenerative phenomena. The high nitrogen requirement of proliferating tumors is one of the characteristics of cancer of interest to the research nutritionist active in this field. The examination of amino acid metabolism in all forms of neoplasia should yield vital data which may aid in their control in the future.*

**The Amino Acid Requirements of the Walker Carcinosarcoma** 256 *In Vitro*. T. A. McCoy, M. Maxwell, and R. E. Neuman. *Cancer Res.* 16: 979, 1956.

Walker carcinosarcoma 256 was grown in T-15 flasks in 2 ml volumes of test medium for 48 hours followed by a change of medium, after which an additional 24 hr of growth was continued. Growth was evaluated by changes in whole cell counts. Inoculations of flasks were quantitatively controlled. A basal medium (consisting of 5 per cent dialyzed human serum in a mixture of 21 amino acids, amides, and vitamins in Earle's solution) was used. Comparisons of growth were made when essential or nonessential amino acids were deleted. Addition of alanine, proline, hydroxyproline, aspartic acid, or glutamic acid to the nonessential amino acid-deficient medium did not significantly increase the growth. Glycine or serine significantly did increase the growth; glycine did so up to a 1–2 mM level, after which inhibition was observed. The inhibition was overcome by adding serine. A combination of both amino acids did not promote growth as well as individual supplementation with serine or glycine.

The Walker 256 tumor was demonstrated to require for *in vitro* growth the twelve amino acids as well as glutamine and asparagine. Eagle has demonstrated the same requirements for the He La tumor. The authors suggested the possibility that glycine inhibition may act as a formyl acceptor in the conversion of inosinic acid to 4-amino 5-imidazole carboxanside ribotide. Observation to support this was that serine overcame glycine inhibition as in transformylating enzyme systems studied by Buchanan. All amino acids tested were inhibitory at a level of 10 to 20 mM. This was interpreted as indicative of amino acid imbalance and/or ammonium toxicity. Interchangeability of glycine and serine is not striking in view of known glycine-serine interconversion and mutual contribution to nucleic acid metabolism. Other workers have already reported that serine was essential for rabbit fibroblasts and could not be replaced by glycine.

The nutritional requirements of tumor tissues are of underlying importance toward a basic knowledge of host-tumor relationships. To what extent do the needs of the neoplasm *in vitro* reflect its requirements *in vivo*? The complexity of *in vivo* experimental design makes the *in vitro* study more feasible. However, in light of the authors' findings it might be well to examine the amino acid requirements of tumor-bearing hosts. To what extent are host nutritive requirements reflec-

tive of tumor needs? Furthermore, the concept of essential and non-essential as applied to amino acids might need to be modified for specific physiologic conditions such as cancer, and specific amino acid supplementation may be a potential adjunct to cancer chemotherapy. E. COHEN

*The use of amino-acid antagonists which block the utilization of specific amino acids by tissues represents an interesting approach to cancer chemotherapy. This type of study began with the anti-folic acid compounds used in the treatment of leukemia. Since the metabolism of the cancer cell is more active than that of normal tissue, the metabolic block imposed by the amino acid analog should have a more significantly depressing effect upon the tumor.*

**The Acute Effects of Beta$_3$-Thienylalanine in the Adult Male Albino Rat.** R. W. Wissler, L. F. Frazier, K. H. Soules, P. Barker, and E. C. Bristow III. *A. M. A. Arch. Pathol.* 62: 62, 1956.

Adult male rats of the Sprague-Dawley strain were tube-fed a maintenance ration in which purified amino acids were used and to which variable amounts of $\beta_3$-thienylalanine were added. The following effects of $\beta_3$-thienylalanine were observed: (1) Body weight and nitrogen balance became depressed within a week and in proportion to the quantity of $\beta_3$-ethylalanine added. (2) There was depression of antibody formation following a single injection of antigen given two days later. Microscopically this manifested itself in decreased proliferation of non-phagocytic, reticular, pyronine-positive cells. (3) Growth of transplanted Jensen sarcoma was retarded, as indicated by decreased mitotic proliferation and decrease of pleomorphic nuclear changes. The $\beta_3$-thienylalanine induced changes were prevented by a diet supplemented with larger amounts of phenylalanine. M. SILBERBERG

*Carbohydrate metabolism in tumor tissue has been shown to proceed at a rate greater than that of normal tissue by some investigators. This may be related to a higher level of energy demand in the form of phosphate bond energy in cancer metabolism. The use of the pentose oxidative route in glucose metabolism by tumor tissue may provide energy with minimum use of the aerobic citric acid cycle.*

**Metabolism of Neoplastic Tissue. IX. An Isotope Tracer Study of Glucose Catabolism Pathways in Normal and Neoplastic Tissues.** C. E. Wenner and S. Weinhouse. *J. Biol. Chem.* 222: 399, 1956.

Catabolism of glucose can take place via at least two pathways: (1) The Embden-Meyerhof process, in which the hexose chain is cleaved symmetrically to trioses, and (2) the oxidative pentose path, involving the oxidation of glucose-6-phosphate to 6-phosphogluconic acid, followed by oxidative cleavage to $CO_2$ and pentose phosphate. Estimations of the relative occurrence of these processes in animal tissues have been attempted by utilizing procedures based on the differential conversion of $C^{14}$-labeled glucose. However, conclusions derived from such experiments are difficult to evaluate.

The present authors employed another procedure for this purpose, which they believe provides a more direct, and hence more reliable, estimation of these processes of glucose catabolism. Their procedure involves the simultaneous incubation of tissue preparations with uniformly labeled, and with 1-, 2-, or 6-labeled glucoses. Isolation and radioactivity assays of intermediates such as acetate, lactate, or related substances allow the calculation of the percentage of such intermediates formed via each pathway.

In this study, glucose catabolism in non-neoplastic tissues of the rat and in solid tumors and suspensions of ascites cells of the mouse, were examined by this method. Lactic acid and respiratory $CO_2$ were collected and assayed for radioactivity.

Analysis of the data for glucose catabolism in non-neoplastic tissues indicates that the Embden-Meyerhof pathway represents the preponderant means of glucose catabolism in the intact rat. Although this is also the predominant pathway for glucose catabolism in tumor tissue, the preponderance of glucose-carbon-1 in the respiratory $CO_2$ over glucose-carbon-6 clearly demonstrates a preferential oxidation of glucose-carbon-1 in all the tumors studied. This indicates that in tumor tissue some glucose catabolism will also take place via the oxidative pentose pathway. M. K. HORWITT

*Ethionine is the ethyl analog of methionine. It interferes with methylation and with protein synthesis. Its effect upon the liver appears to depend upon experimental conditions employed; in one report, it exerted a lipotropic effect and, in another study, fatty livers resulted from its administration. The production of hepatic carcinoma by this agent may be related to the occurrence of this disease in humans with longstanding portal cirrhosis.*

**Carcinoma of the Liver in Rats Fed Ethionine.** E. Farber. *A.M.A. Arch. Pathol.* 62: 445, 1956.

Young male and female Wistar rats received a semi-synthetic diet containing 0.25 per cent DL-ethionine for periods of eight to nine months. Of fourteen animals twelve developed carcinomas of the liver, eleven of the liver cell type; seven animals had metastases to lymph nodes, omentum, pancreas, mediastinum, and lungs. M. SILBERBERG

## CHOLESTEROL LEVELS AND METABOLISM

*Among the factors controlling serum cholesterol levels at fairly constant values for individual subjects are the rate of endogenous formation of the molecule in the liver, the dietary cholesterol and fat intakes, humoral factors, the rate of excretion of oxidative products in feces, and the activity of the reticulo-endothelial tissues in removing cholesterol from the blood. In most subjects, the rate of production and absorption are balanced by the rate of disposal, resulting in a sort of cholesterol homeostasis*

*Changes in the blood level of cholesterol will reflect an alteration in one or more of the factors controlling the homeostatic balance.*

**Role of Reticulo-Endothelial System in Blood Clearance of Cholesterol.** T. Neveu, G. Biozzi, B. Benacerraf, C. Stiffel, and B. N. Halpern. *Am. J. Physiol.* 187: 269, 1956.

The clearance rate of colloidal carbon particles is only slightly impaired in rabbits rendered hypercholesteremic by overfeeding cholesterol. Rabbit serum cholesterol injected into rats is cleared by phagocytosis of the reticulo-endothelial cells whether the cholesterol is in the form of chylomicra (cloudy serum) or not (clear serum from fasting animals). The clearance of the foreign serum cholesterol from the blood follows an exponential function with respect to time as for many other colloids and the rate of clearance is inversely proportional to the quantity injected. Interference is observed between the clearance of cholesterol and other colloids when injected simultaneously. Pretreatment of rats with high doses of heparin increases substantially the speed of clearance of foreign serum cholesterol.

AUTHORS

*While changes in thyroid activity do not appear to affect the absorption of cholesterol, it has been shown that the rate of disposal of cholestero' is directly related to thyroid function. The hypercholesterolemia of hypothyroidism is probably the result of reduced excretion and/or reticulo-endothelial uptake of this substance.*

**Effect of Hyper- and Hypothyroidism on Intestinal Absorption of Cholesterol in Rats.** R. H. Rosenman and M. Friedman. *Am. J. Physiol.* 187: 381, 1956.

Thoracic duct lymph was collected from normal and hyper- and hypothyroid rats following administration of a test dose of cholesterol dissolved in olive oil. The data indicate that such deranged thyroid function does not significantly alter the intestinal absorption of cholesterol.

AUTHORS

*The role of sulfur in cholesterogenesis may involve the intermediary metabolism of acetate where energy carrying sulfur compounds are active.*

**Effect of Diets Low in Organic Sulfur on Metabolism of Cholesterol-$C^{14}$ in the Rat.** O. W. Portman. *Am. J. Physiol.* 186: 403, 1956.

Mechanisms involved in the hypercholesteremia of rats fed diets relatively deficient in organic sulfur were investigated. Acetate-$C^{14}$ was injected into rats fed diets including alpha-protein and into methionine-supplemented rats. The rates of synthesis and elimination of cholesterol-$C^{14}$ from tissues were studied. The biological half lives of cholesterol-$C^{14}$ in all tissues were similar for both diet groups. The rate of incorporation of acetate into cholesterol was somewhat less in the livers of rats from the deficient group at 40 minutes after injection of the acetate. The ratio of cholesterol to fatty acid synthesis was similar for the two groups. The mean acetylation of a test dose of *p*-aminobenzoic acid was almost identical for the two groups.

AUTHOR

**Rape Oil and Cholesterol Metabolism in Different Species with Reference to Experimental Atherosclerosis.** K. K. Carroll. *Proc. Soc. Exp. Biol. & Med.*, 94: 202, 1957.

Rats fed rape oil or erucic acid showed deposits of cholesterol in the adrenals and liver. These substances had however little effect on the level of blood cholesterol or on atheroma development. Since rats are very resistant to atherosclerosis anyhow, similar experiments were carried out on species susceptible to experimental arteriosclerosis. Rape oil had little effect on adrenal cholesterol of rabbits, guinea pigs, chickens and dogs, especially if compared with the marked atherogenic effect of cholesterol.

M. SILBERBERG

*The gradual and unexplained rise in serum cholesterol with increasing age has been demonstrated in several geographic areas and at various socio-economic levels. Since physical acivity has become a factor of recognized importance in the control of serum lipid levels, one might postulate that the more sedentary habits of aging individuals is accountable for the rise in serum cholesterol.*

**Serum Cholesterol Levels of Young and of Elderly Women Consuming an Institution Diet.** G. R. Walker, E. H. Morse, and M. Potgieter. *J. Nutrition* 60: 517, 1956.

The subjects for this study were 29 women in a state training school for the handicapped; all were in good physical health. They were divided into two groups according to age. In the younger group of 15 subjects the average age was 31 years, with a range of 28 to 34. In the older group of 14 subjects the average age was 64 years, with a range of 56 to 77. During the four months of the study the subjects ate together and all were served the usual institution diet. Calculations showed that, with the exception of iron, their diet on the average contained at least 100 per cent of the daily allowances for nutrients recommended by the National Research Council. It contained over 96 per cent of the iron allowance. As these women were also serving as subjects in an ascorbic acid study, the high-vitamin C foods, such as citrus fruits, tomatoes, pineapple, and raw cabbage, were omitted from their diet. Their diet was restricted in ascorbic acid to an average daily intake of 32 mg, but was adequate in all other nutrients. Samples for serum cholesterol analysis were taken at the end of the period of restricted diet and following supplementation of the diet with 25, 50, and 75 mg of ascorbic acid.

The mean serum cholesterol level for the older women was 230 ± 9 mg per 100 ml of serum and for the younger, 172 ± 8 mg. The correlation between age of subjects and serum cholesterol level was positive and

significant at the 1 per cent level. Calculations from the 16-day food records showed that the two groups consumed very nearly the same amounts of all nutrients. Results of this study indicate that the higher serum cholesterol levels found in older women were related to age rather than to diet. A slight rise in serum cholesterol with rise in serum ascorbic acid was noticed in the older group, but not in the younger group. The correlation between serum cholesterol and percentage deviation from desirable weight was not significant.

B. SURE

*The use of EDTA to remove calcium from arteriosclerotic vessels has recently been reported as resulting in significant improvement in patients with coronary artery disease. In the present study, it has been employed to lower plasma cholesterol. The effects of long-term administration of the chelating agent are as yet unknown so that this method of treatment cannot be recommended for general use.*

**A Practical Method for the Reduction of Plasma Cholesterol in Man.** H. A. Schroeder. *J. Chronic Dis.* 4: 461, 1956.

The author describes what he calls a practical method for the reduction of plasma cholesterol in man. The method employs a diet fairly low in saturated fatty acids but providing an adequate intake of linolenic acid, a metal chelator (oral EDTA), and pyridoxine. This was carried out on outpatient basis.

As the author points out the results were variable, but in some patients there was a considerable lowering of the plasma cholesterol. The rationale of the program is discussed. "The diet without chelating agent or pyridoxine was usually not associated with profound falls" (plasma cholesterol). The chelating agent (EDTA) was used because of the observation that when given intravenously it lowered plasma cholesterol. The use of pyridoxine was empirical.

This should be regarded as a preliminary study conducted on an outpatient department basis. It needs to be confirmed by a longer and larger study. A word of caution should be given regarding the use of EDTA in that one of the toxic reactions may be the nephrotic syndrome.

K. R. CRISPELL

## ASCORBIC ACID

*The specific biochemical reactions in which vitamin C is involved in cellular metabolism have not been clearly defined. The high oxidation-reduction potential of the vitamin has been shown to be of importance in l-tyrosine and phenylalanine transformations and in the conversion of folic acid to folinic acid. However, its mechanism of action as a possible coenzyme remains uncertain. The clinical aspects of ascorbic acid deficiency, on the other hand, have been carefully delineated. The pathologic and physiologic changes associated with a deficient intake of this agent are well known. Nevertheless, investigators in this field have difficulty in relating the current biochemical knowledge of the vitamin to pathophysiology of ascorbic acid deficiency. Growth retardation during periods of vitamin C deficiency is an intriguing observation which may be related to several of the actions of the vitamin.*

**The Nutrition of Nigerian children, with Particular Reference to their Ascorbic-Acid Requirements.** B. M. Nicol. *Brit. J. Nutrition* 10: 275, 1956.

A study of the diets eaten by 10 to 12-year-old children of Ibo and Yoruba salaried officials living in Kaduna, Northern Nigeria, was undertaken in 1954. The results confirmed an impression that the customary diet of these children supplies only small amounts of ascorbic acid during ten months of the year owing to a shortage of fresh fruit and vegetables, and that large quantities of vitamin C are obtained from mangoes during the two months beginning about mid-April. The investigation reported in this paper was designed to record the heights and weights of such children throughout one year, and to study the effect on growth of supplementing their diet with ascorbic acid.

Two groups of 40 Nigerian children 9 to 15 years of age, resident in Kaduna, were paired for age, sex, and height. Their customary diet, which is described, supplied about 16 mg ascorbic acid daily for ten months of the year, the amount being increased to 170 mg by the consumption of mangoes during the remaining two months. The diet of one group was supplemented throughout the year with ascorbic acid in two different amounts, and a control group received tablets made of lactose, stearic acid, essence of lemon and tartaric acid. During the experimental period of one year the average gain in height of the children in the group treated with ascorbic acid was significantly greater than that of the untreated group, but body-weight was unaffected by the vitamin C supplement.

The cristal height, pelvic intercristal diameter and antero-posterior and transverse thoracic diameters of the children were measured. The gain in cristal height and intercristal diameter during the year was greater in the treated than in the untreated group, but not significantly so. The clinical features noted at examination toward the beginning and at the end of the experimental period are described.

The concentrations of ascorbic acid in the plasma at different levels of intake were determined. A significant correlation was found between intake and plasma concentration of individuals up to 160 mg and 0.73 mg/100 ml, respectively, but was absent at values of 230 mg and 0.97 mg/100 ml. The erythrocyte sedimentation rate was found to be significantly lower in the treated group than in the untreated group. It is suggested that the children's average daily intake of ascorbic acid was not sufficient to maintain the maximum growth rate which a diet of the pattern described would otherwise support.

B. SURE

*Vitamin C levels in plasma are considered optimal at a range of 0.8 to 1.0 mg/100 ml. The buffy coat levels of vitamin C under conditions of normal vitamin C intake are in the range of 25 mg/100 ml. However, in the presence*

*of much lower levels of vitamin C in tissues and plasma no evidence of scorbutic tendencies have been manifested.*

**Ascorbic Acid Utilization by Women. Response of Blood Serum and White Cells to Increasing Levels of Intake in Two Groups of Women of Different Age Levels.** E. H. Morse, M. Potgieter and G. R. Walker. *J. Nutrition* 60: 229, 1956.

Two groups of women, patients at a state training school and hospital for the handicapped, served as subjects during the winter of 1954 to 1955. The average age of fifteen was 31 years (range 28 to 34) and thirteen were of an average age of 64 years (range 56 to 77). All were in good physical health, and were mentally capable of co-operating in the study. The subjects were examined at the beginning and end of the study for clinical signs of vitamin C deficiency.

Following seven weeks on a 32 mg intake of ascorbic acid, the average serum ascorbic acid level of 15 young women was 0.33 mg per 100 ml. It rose to 1.76 mg during a period of ten weeks in which the ascorbic acid intake was increased gradually to 107 mg per day. The average white cell ascorbic acid rose from 25.6 mg per 100 g of white cells to 35.2 mg during the first four weeks when the intake had reached 57 mg per day, and thereafter remained stationary.

The average serum ascorbic acid level for 13 older women, on the same levels of intake, rose from 0.24 to 1.42 mg during the 10-week period. The average white cell ascorbic acid rose from 22.2 to 34.9 mg during the first six weeks when the intake had reached 72 mg per day, and thereafter remained stationary.

Correlation between serum and white cell ascorbic acid levels was significant only in the young group on intakes of 32 mg and 47 mg of ascorbic acid per day. There was no significant difference in uptake of ascorbic acid by the white cells in the young group as compared with that of the older group. Difference in regression of white cell level on serum level between the two groups was not significant. B. SURE

*The normal processes involved in the maturation of ground substance is an uncharted area. The need for vitamin C in the formation of collagen fibrils within the mucopolysaccharide matrix is demonstrated by the failure of wounds to heal or bone matrix to develop normally in deficiency states involving this agent. The lack of uptake of sulfate in scorbutic tissues and accumulation of hyaluronates suggests a block in sulfation of the mucopolysaccharides elaborated by the fibroblasts within connective tissue.*

**Polysaccharide Formation in Repair Tissue During Ascorbic Acid Deficiency.** W. van B. Robertson and H. Hinds. *J. Biol. Chem.* 221: 791, 1956.

The data presented in this paper show that excessive amounts of mucopolysaccharides accumulated in repair tissue formed in the absence of adequate ascorbic acid. Granulomatous repair tissue was induced in guinea pigs by subcutaneous injection of an extract of Irish moss. The collagen-poor tissue so formed contained five times as much mucopolysaccharides as repair tissue similarly induced in animals receiving adequate levels of ascorbic acid. The predominant polysaccharide which accumulated in scorbutic repair tissue was isolated and identified as hyaluronic acid.

M. K. HORWITT

**Labeled Sulfate in Scorbutic Guinea Pigs.** A. C. Upton and T. T. Odell Jr. *A.M.A. Arch. Path.* 62: 194, 1956.

In young adult guinea pigs kept on a scorbutic diet for 12 days, fragments of extensor muscle from the thigh were excised. Four days later, the animals were injected with 170 μc/kg body weight of $S^{35}$-labeled sodium sulfate in 0.3 ml saline. Tissue repair was studied histologically and autoradiographically. In scorbutic animals little $S^{35}$ had been taken up as compared to large amounts found in the non-scorbutic controls. The sulfate was deposited predominantly outside the cells at the site of fiber formation. In megakaryocytes and platelets of the scorbutic animals, the uptake of $S^{35}$ was slightly depressed. There was no uptake of radiosulfate by immature fibroblasts including those containing PAS-positive material.

M. SILBERBERG

*It is difficult to accept the hypothesis that vitamin C deficiency plays a role in the production of diabetes. The oxidation product of vitamin C, dehydroascorbic acid, has been shown experimentally to exert a diabetogenic effect.*

**Histopathological Changes by Acetoacetate in Normal and Scorbutic Guinea Pigs.** S. G. Nayudu, S. Mookerjen, and M. C. Nath. *Proc. Soc. Exp. Biol. & Med.* 93: 179, 1956.

Acetoacetate which induces diabetes and depletes the stores of thiamine, riboflavin, niacin and vitamin C was injected into normal and scorbutic young adult male guinea pigs daily for 21 days in concentrations increasing from 175 mg/kg to 415 mg/kg. The pancreatic islets of scorbutic guinea pigs and of those injected with acetoacetate were hypertrophic. Injections of the acetate into scorbutic guinea pigs caused further degranulation and degeneration of the islet cells. Both scurvy and acetoacetate caused central degeneration of the liver, the change was enhanced by combined treatment. It is claimed that acetoacetate may be a prime factor in the development of clinical diabetes. M. SILBERBERG

*The following abstracts deal with an abortive effort to solve the problem of treatment of the common cold with ascorbic acid and flavonoids.*

**Usefulness of Bioflavonoids and Ascorbic Acid in Treatment of Common Cold.** H. E. Tebrock, J. J. Arminio, and J. H. Johnston. *J.A.M.A.* 162: 1227, 1956.

In this study on the usefulness of bioflavonoids and ascorbic acid in the treatment of common colds, a large number of patients (about 2,000) were studied at several industrial outpatient clinics. To these were added a number of college students and private patients. A single medical check up three days after the development of a cold was utilized. A good experimental design was used, and information was analyzed by means of coded punch cards.

There was a singular lack of effect produced by either the bioflavonoids (250 mg of a lemon extract) or ascorbic acid, either alone or together. No statistically significant differences could be obtained. The total dose received by the patients was 200 mg of ascorbic acid and one gram of the bioflavonoid a day when symptoms appeared, yet there was no effect on subjective or objective manifestations. S. O. WAIFE

**Blood Ascorbic Acid Level in Bioflavonoid and Ascorbic Acid Therapy of Common Cold.** W. L. Franz, W. Sands, and H. L. Heyl. *J.A.M.A.* 162: 1224, 1956.

There have been reports that bioflavonoids may prevent or modify the common cold. This study was made on 89 volunteer medical students and student nurses during the winter and early spring of 1956 under the experimental scheme of a "double blind" technic. Four groups received in rotation the following capsules: (1) a placebo, (2) a bioflavonoid and ascorbic acid, (3) ascorbic acid alone, and (4) the bioflavonoid alone (an extract of grapefruit peel). The subjects were supposed to take three capsules a day for three months. Close supervision and co-operation seemed to keep this source of error down to a minimum.

The administration of the bioflavonoid had no effect either on the incidence or cure of colds or on the ascorbic acid level of the blood. S. O. WAIFE

## DIABETES MELLITUS AND THE SULFONYLUREAS

*Reports dealing with the clinical usefulness and sites of action of the sulfonylurea compounds are flooding the current medical literature. Two excellent symposia have been published* (Metabolism, *Nov. 1956;* Diabetes, *Jan.–Feb. 1957*) *in which the results of animal experimentation and clinical trials with these agents are thoroughly discussed. Lacking in these reports are adequate evaluations of the effect of long-term administration of the drugs upon human subjects or upon animals. Such observations are essential over long periods before general use of these agents can be recommended. The compound, carbutamide, which was shown to have a potent antidiabetic action, has been withdrawn from further clinical trial because of a significant incidence of side-effects ranging from 6 to 11 per cent. These toxic effects were believed to account for eight deaths attributed to myocarditis, hepatitis and sulfonamide sensitivity* (Diabetes *6: 91, 1957*).

*It is apparent that these compounds have no effect upon blood glucose levels in the absence of insulin. Their mechanism of action is not known. Two concepts are receiving support currently: (1) stimulation of insulinogenic function of the beta cells of the pancreatic islets, and (2) inhibition of glucose liberation from liver by reduction of glucose-6-phosphatose activity. The present experience indicates that the clinical value of these compounds is confined to the older person suffering from mild diabetes in whom insulin-producing tissue is present. If stimulation of the beta cells can be shown to represent the mode of action of these compounds, the question must be raised as to whether they can ultimately exhaust the insulin-producing mechanism.*

**Hypoglycaemic and Antidiabetic Sulphonamides.** F. G. Young. *Brit. Med. J.* 1956, 2: 431.

The history of antidiabetic sulfonamides is briefly traced from the chance observation in wartime France that hypoglycemia sometimes occurred during the treatment of typhoid fever with a sulfonamide, to the discovery of N-butyl-N-sulphanilylurea, BZ-55. BZ-55, with its related compounds, according to these authors, appears to be a non-competitive inhibitor of liver insulinase. It also is said to stimulate the secretion of insulin by the $\beta$-cells of the pancreatic islets.

It has been shown that acridine possesses hypoglycemic activity and that in some circumstances penicillin has an antidiabetic action. There is evidence to show that normal tissue, particularly the liver, has a considerable bacterial content and the question is posed "is it not possible that the breakdown of insulin in the normal or in the diabetic animal might result from the activity of bacteria present in normal tissues?" The suggestion is also made that the stimulation of insulin secretion might be caused by the stimulation of the $\alpha$-cells to produce glucagon or other hyperglycemic substances. F. E. HYTTEN

**A Clinical Trial of BZ-55.** L. J. P. Duncan, J. D. Baird, and D. M. Dunlop. *Brit. Med. J.* 2: 433, 1956.

The effect of the antidiabetic sulfonamide BZ-55 was tested in 44 patients over the age of 45 and overweight by not more than 10 per cent of their ideal weight. Experience had suggested that it was of little value in young people with diabetes. The 44 patients tested could not be controlled by dietary measures alone and they experienced diabetic symptoms in the absence of insulin; in 32 of them the drug was a successful "insulin substitute." It was given in initial doses of from 3 to 4.5 g and then reduced gradually to 1.5 g daily. It is without effect on ketosis or on patients who readily become ketotic; suitable subjects are to be found only by trial and error. There was a high incidence of toxic effects: skin reactions, fever and slight granulopenia, particularly thrombocytopenia which was associated with purpura in two cases.

The character of the hypoglycemic effect was studied with particular reference to the daily profile of glycemia, glucose tolerance and its relationship to fasting blood

sugar levels. BZ-55 has a remarkable hypoglycemic effect on some patients, apparently due to an average reduction of about 40 per cent in the fasting blood-glucose level. Glucose tolerance is unchanged and there is no alteration in the daily profile of glycemia, although the absolute values are set at a lower level and glycosuria is reduced.

"BZ-55 will almost certainly prove to be a valuable research weapon which may help to elucidate some of the obscure problems of diabetic metabolism. Nevertheless, we believe that it would be premature at the present time to make it generally available for therapeutic use." F. E. HYTTEN

**Trial of an Oral Hypoglycaemic Agent in Diabetes.** F. W. Wolff, G. A. Stewart, M. F. Crowley, and A. Bloom. *Brit. Med. J.* 2: 440, 1956.

The response to the oral administration of BZ-55 was studied in 45 patients with diabetes usually requiring insulin. They were inpatients under careful control.

Clinical success was achieved in 19 cases. These patients were on the whole older than those in whom this therapy failed and they had a shorter history of the disease. Often they had a history of diminishing insulin requirements. Their fasting blood sugar ranged from 123 to 275 mg/100 ml. Those who were not successfully controlled by the drug had a higher average insulin requirement, their fasting blood sugar ranged from 128 to 421 mg/100 ml and they had a tendency to acetonuria.

BZ-55 exerted no control over the post-prandial rise of blood sugar in either group. "Its action is therefore probably on the mechanism controlling the utilization of endogenous glucose." The body weight apparently did not influence the success or failure of the drug.

There was no difference in the levels of free and conjugated sulfonamides in the blood of successes and failures. In the urine there was more excretion of conjugated sulfonamides in the unsuccessful cases than in the successful ones. Five patients developed skin reactions to the drug. There were no other complications.
F. E. HYTTEN

**BZ-55 in Diabetes.** J. M. McKenzie, P. B. Marshall J. M. Stowers, and R. B. Hunter. *Brit. Med. J.* 2: 448 1956.

Results are reported of the use of BZ-55 on 20 patients with diabetes. Ten cases were mild, but not satisfactorily controlled by diet alone. They were on a standard diet while in the hospital. Detailed investigations were made of blood and urinary sugar and the blood level of BZ-55. Three similar subjects were treated as outpatients; another three patients who required insulin to be free of ketosis were also treated and in a fourth group of four patients BZ-55 was given in addition to insulin.

The findings are described briefly. The numbers are too small to permit any firm conclusions but the following seems likely: BZ-55 has a "normalizing" effect on the blood sugar of patients with mild cases of diabetes, the higher the fasting blood sugar, the greater the effect. The effect does not seem to be due to an increased sensitivity to insulin. There was no correlation between sensitivity to BZ-55 and the duration of the diabetes. The four patients receiving BZ-55 in addition to insulin had better control than when receiving insulin only. There were toxic reactions in four subjects. Two had widespread skin rashes and two suffered from nausea. F. E. HYTTEN

**Hypoglycaemic Sulphonamides in Treatment of Diabetes.** G. Walker, W. L. B. Leese, and J. D. N. Nabarro. *Brit. Med. J.* 2: 451, 1956.

The effect of BZ-55 was tested on 24 "stable" middle aged persons with diabetes. The age range was 38 to 82 years. Six of these patients were receiving insulin and all but two had glycosuria. None was ketotic. The BZ-55 was given in doses of 2.5 and 1.5 g for the first two days, 1.0 g daily thereafter.

In 23 of the patients there was a drop in the blood sugar which remained at a satisfactory level, and in only one of these was there residual glycosuria. The twenty-fourth patient, in spite of 2 g of BZ-55 daily had a blood sugar of 238 mg/100 ml, possibly due to poor dietary control. Two patients developed a rash and one became hypothyroid. Another developed a marked neutropenia after treatment for 10 weeks.
F. E. HYTTEN

**The Value of BZ-55 in Control of Diabetes.** I. Murray and I. Wang. *Brit. Med. J.* 2: 452, 1956.

A trial of BZ-55 in 25 patients with diabetes is reported briefly. The majority were treated as outpatients. Dosage was 2.5 g and 1.5 g for the first two days and 1 g thereafter; it was preceded by two weeks' treatment by placebo tablets.

There was very successful control of blood sugar and glycosuria in 11 out of 23 of these patients, all of average weight and over 45 years of age. In some cases the response was dramatic, glycosuria disappearing within 24 hours. None had previously taken insulin. In the other 12 patients, six were partially successful, one of these had had insulin; and the other six, all of whom had had insulin, failed to respond. In the remaining two "younger" patients, the drug was completely ineffective. BZ-55 proved ineffective in two patients while they were suffering from sepsis, but it produced a considerable effect when this was overcome. No toxic effects are reported. F. E. HYTTEN

**Clinical Trial of the New Oral Hypoglycaemic Agent BZ-55.** J. A. Hunt, W. Oakley, and R. D. Lawrence, *Brit. Med. J.* 2: 445, 1956.

Two groups of patients were treated; 21 inpatients covering a wide range of age, weight and severity of disease and 17 outpatients selected on the basis of experience with the 21 inpatients. There was rigid dietary control; in the first two days 2.5 and 1.5 g of BZ-55

was given and on subsequent days the dosage was reduced to 1.0 g.

In general it appeared that young people with diabetes and those requiring more than small amounts of insulin showed little response even to the extent of the reduction of the insulin dosage. The presence of ketonuria was consistently associated with complete failure to respond to BZ-55. An interesting case was a nine-year-old girl who had had diabetes for five months and who had no ketonuria and had never had insulin; she showed no response to BZ-55 Eight cases felt giddiness after taking the drug, three had a drug rash and one a fever. F. E. HYTTEN

**Effect of Carbutamide on Serum Cholesterol Level in Diabetes Mellitus.** I. B. Munro and D. Murray, *Lancet* 2: 1083, 1956.

Changes in serum cholesterol were noticed during a clinical trial of carbutamide (BZ-55) in the treatment of diabetes mellitus. The effect has been studied in six subjects, four of whom showed the characteristic picture of a fall in serum cholesterol during the first days of treatment followed by a fairly rapid rise to previous levels in spite of continued treatment. Two such cases are described in detail.

In two normal subjects, carbutamide caused no disturbance of serum cholesterol. The change could not be induced in the diabetics by insulin alone. The authors pose two questions: "Does this change in the serum cholesterol reflect, then, some fundamental difference in the types of diabetes studied? Or is it evident that, compared with insulin, carbutamide has a different (or at least more widespread) effect on intermediary metabolism?" F. E. HYTTEN

**The Use of Some Sulfamide Compounds in the Treatment of Experimental Diabetes Mellitus.** A. Loubatieres. *Presse Med.* 63: 1701, 1728, 1955.

In 1942 the author reached the conclusion that compound 2254 RP stimulates the secretion of the islands of Langerhans. In a normal dog this compound causes symptoms of hypoglycemia. It is inactive in a depancreatized dog. Compound 2254 RP also relieves the severity of alloxan diabetes in rabbits. The author believes that it stimulates the beta cells, and, under certain circumstances, the alpha cells. Thus *p*-aminobenzene sulfamidoisopropylthiodiazol would seem to be active on the pancreas. H. GOUNELLE

**Metabolic Effects of Carbutamide in Selected Diabetics.** P. A. Bastenie, J. R. M. Franckson, R. De Meutter, T. C. Damanet and V. Conard. *Lancet*, 1: 504, 1957.

Treatment of diabetes with the sulfonamide, carbutamide (BZ-55) has been largely empirical since its mode of action is not known. Experiments were performed on 18 subjects: Of these sixteen were between 42 and 82 years of age and were of the obese middle-aged type and had never required much insulin. One other patient had had diabetes for 20 years and required a high insulin dosage and the other, age 26, had mild diabetes which had been recently discovered.

Intravenous glucose tolerance tests and insulin tests were made and the serum levels of lipids, cholesterol and protein were estimated before treatment with carbutamide, after initial treatment, and after prolonged treatment.

The results showed no increase in glucose uptake by the tissues and no insulin-sparing action was demonstrable. The high output of glucose by the liver in insulin-induced hypoglycemia, which is characteristic of diabetes, is reduced, possibly due to inhibition of hepatic glucose-6-phosphatase by the drug. Less protein and fat are catabolised. F. E. HYTTEN

**Intrapancreatic Perfusion of the Antidiabetic Sulfonylureas.** A. R. Colwell, Jr., J. A. Colwell and A. R. Colwell, Sr.; *Metabolism* 5: 749, 1956.

Infusion of carbutamide or tolbutamide in small amounts (7 to 71 mg/kg) into the pancreatic arteries of normal anesthetized dogs resulted in hypoglycemia. Similar infusions into the portal vein caused a hyperglycemic response. The blood levels obtained with pancreatic artery administration of the compounds were much below the levels observed during treatment. These data suggest that the sulfonylurea compounds act by a pancreatic mechanism, probably an accelerating release of insulin from beta cells.

C. R. SHUMAN

**The Action of a Sulfonylurea Hypoglycemic Agent in Extrahepatic Tissues.** A. N. Wick, B. Britton and R. Grabowski. *Metabolism* 5: 739, 1956.

A sulfonylurea hypoglycemic compound bearing an $S^{35}$ label was injected into experimental animals in order to investigate its extrahepatic effects upon glucose metabolism. The compound was found to circulate only within a volume equivalent to the extracellular compartment of the extrahepatic tissues. This distribution was unchanged by maximal insulin injections. The compound did not influence the rate of oxidation of glucose as measured by $C^{14}O_2$ recovery after the administration of $C^{14}$-labeled glucose. It did not accelerate the intracellular transfer of glucose into muscle cells. It was concluded that the sulfonylurea compound had no direct effect upon extrahepatic muscle tissues. C. R. SHUMAN

**Studies on the Site of Action of the Arylsulfonylureas in Man.** A. E. Renold, A. I. Winegrad, E. R. Froesch and G. W. Thorn. *Metabolism* 5: 757, 1956.

Diabetic patients were studied by means of metabolic balance data and comparative intravenous glucose, fructose and galactose tolerance tests before, during, and after therapy with carbutamide and tolbutamide. Inhibition of $I^{131}$ uptake was demonstrated with use of carbutamide; however, the hypoglycemic effects of these agents could not be attributed to any alterations

in pituitary, adrenal, or thyroid function. No peripheral insulin-like effect could be demonstrated as shown by a failure of the compounds to significantly influence the rate of blood glucose decline after a glucose load, the absence of an effect on serum or urine phosphate, urinary nitrogen excretion or on blood lactate or pyruvate. These drugs were found to decrease the rate of conversion of galactose or fructose to glucose. These findings suggest either a decrease in glucose synthesis or release and tend to implicate glucose-6-phosphatase since it is involved in the reactions necessary for the conversion of these hexoses into glucose. Selective interference with gluconeogenesis may contribute to the hypoglycemic action of these agents.

C. R. SHUMAN

## VITAMIN $B_{12}$: HEPATIC AND NEURAL EFFECTS

*In addition to its anti-pernicious anemia action, vitamin $B_{12}$ exerts an effect upon the metabolism of other tissues. Studies on the biochemical activity of this factor indicate its role in protein synthesis and, to a lesser extent, in lipid and carbohydrate metabolism. The formation of ribose from glucose is reduced in vitamin $B_{12}$ deficiency; impaired glycolysis has also been reported and may be due to diminished cellular glutathione levels. The derangement of lipid metabolism has been attributed to a reduction in available methionine and to impaired glycolysis observed in vitamin $B_{12}$ deficiency states. The clinical use of vitamin $B_{12}$ in hepatic disease has given rise to conflicting reports. Evidence favoring its lipotropic effect in hepatic injury in experimental animals is reported.*

**Protective Action of Vitamin $B_{12}$ in Experimental Liver Injury.** D. K. Kasbekar, D. V. Rege, and A. Sreenivason. *Nature* 178: 989, 1956.

Two groups of rats were given 2 cc/kg body weight of carbon tetrachloride to produce liver damage. One group had been given 10 $\mu$g/animal of vitamin $B_{12}$ three hours previously, the other group acted as controls.

The livers were examined 6 and 48 hours after poisoning. Total lipid content rose in the damaged livers, but less in those protected by vitamin $B_{12}$. Vitamin $B_{12}$ prevented an initial fall in glutathione and reduced the fall in phospholipid and ribonucleic acid content. It had no effect on the fall in glycogen content of the damaged liver. Desoxyribonucleic acid and methionine values were unchanged throughout.

F. E. HYTTEN

*In hepatic disease, the absorption of vitamin $B_{12}$ may be increased. Serum levels of the vitamin are elevated; the serum $B_{12}$-binding substances appear to be decreased. Furthermore, the urinary excretion of the vitamin is low in hepatic disease. Experiments have been reported implicating $B_{12}$-binding substances as inhibitors of vitamin $B_{12}$ absorption. It is possible that in liver disease a reduced capacity for the formation of the binding substance enhances the rate of absorption of the vitamin.*

**Vitamin $B_{12}$ Excretion as Index of Hepatic Disorder.** H. Baker, I. Pasher, H. P. Dolger, and H. Sobotka. *Clin. Chem.* 2: 328, 1956.

Bioassays for vitamin $B_{12}$ by three independent systems employing *Lactobacillus leichmanni*, *Euglena gracilis*, and *Ochromonas malhamensis* were compared with the clinical diagnosis of twenty-three patients, three of whom were normal controls. This is a followup of an earlier study in which all diabetic patients who excreted only 20 per cent of intramuscularly administered vitamin $B_{12}$ were found to have had a history of liver disease or hepatic insufficiency.

In this study, thirteen of the reported cases with a clinical diagnosis of liver involvement showed increased absorption of vitamin $B_{12}$ after administration of a 50 $\mu$ intramuscular dose. Two cases considered clinically convalescent still did not show normal excretion of the vitamin. Four convalescent pneumonia patients were included who showed lower excretion of the vitamin than did the controls. This was considered as indicative of the effect of infection upon the liver. Seven cases including one with neurologic disease excreted 25 to 40 $\mu$g of the load dose. However, none of the patients with liver disease excreted more than 10 $\mu$g of the load dose as measured by the *Euglena* and *Ochromonas* assays; the *Lactobacillus* assay method appeared more conservative.

The authors are extending their studies. It is hoped that future reports will correlate their assay data with the results of conventional liver function tests. Notwithstanding, measurement of the excretion of a normal metabolite as vitamin $B_{12}$ may prove valuable as another criterion of the degree of convalescence from liver disease.

E. COHEN

**Serum Vitamin $B_{12}$ Level and Binding Substance of Tuberculous Patients With and Without Liver Disease.** R. L. Davis, R. C. Duvall, and B. F. Chow. *J. Lab. & Clin. Med.* 49: 422, 1957.

The authors present further studies on the uptake of radioactive vitamin $B_{12}$ labeled with cobalt$^{60}$ by the resting cells of *Lactobacillus leichmannii*. They had previously reported that certain biologic compounds such as heparin had the capacity to prevent uptake by these cells. This present communication deals with serum vitamin $B_{12}$ levels and binding substance of tuberculous patients with and without liver disease.

The binding level in 15 patients with tuberculosis and infectious hepatitis was found to be markedly decreased. The authors also present evidence that there appeared to be an inverse relationship with serum bilirubin. As has been reported previously, the serum level of vitamin $B_{12}$ is very high during the acute stage of hepatitis. C-reactive protein was measured and there was no correlation between this level and the binding capacity of the serum for vitamin $B_{12}$. As the

hepatitis subsided and the serum bilirubin returned to normal limits the binding capacity of the serum for vitamin $B_{12}$ rose to normal limits.

From the data presented it is not possible to decide whether the primary factor in decreased binding was the high level of vitamin $B_{12}$ in the serum or the elevated level of bilirubin. The study of cirrhotics suggests that it is the latter as these patients maintain their binding capacity despite a high level of vitamin $B_{12}$ in the serum. K. R. CRISPELL

**Serum Concentrations of Vitamin $B_{12}$ in Acute and Chronic Liver Disease.** M. Rachmilewitz, J. Aronovitch, and N. Grossowicz. *J. Lab. & Clin. Med.* 48: 339, 1956.

The authors present studies on the serum level of vitamin $B_{12}$ in acute and chronic liver disease. The vitamin $B_{12}$ determinations were carried out by a modified *E. coli* assay. The maximum binding capacity of the serum for vitamin $B_{12}$ was also determined.

Thirteen of fourteen patients with acute viral hepatitis had definite elevation for above the normal range. The highest values were found in the early phase of the disease before blood bilirubin reached the highest level. In cirrhosis the findings were less uniform but the highest values were found in the patients with severe liver damage. In six cases of proven extrahepatic obstruction the serum level was normal as were liver function tests.

The mechanism responsible for the increased elevation of vitamin $B_{12}$ is unexplained. Increased binding by serum proteins, decreased excretion, and release of the stored vitamin from the damaged liver were suggested as the possible mechanism.

This is an interesting observation and needs confirmation in a large group of patients. K. R. CRISPELL

*In the metabolism of neural tissue, it is probable that vitamin $B_{12}$ exerts an effect through its action upon the formation of ribose and upon lipid metabolism. It may be presumed also that the formation of labile methyl groups is necessary for the formation and maintenance of the myelin sheath. Vitamin $B_{12}$ also plays a vital role in the transfer of labile methyl groups. In terms of its biochemical actions, the consequences of vitamin $B_{12}$ deficiency in relation to cerebral and peripheral nerve tissues is understandable. In experimental animals depleted of vitamin $B_{12}$ distinct histopathologic alterations of the central and peripheral neuronal structures have been described.*

**Cerebral Manifestations of Vitamin $B_{12}$ Deficiency.** J. M. Holmes. *Brit. M. J.* 2: 1394, 1956.

Although it has long been recognized that encephalopathy occurs commonly in vitamin $B_{12}$ deficiency, it is much less well known than the spinal and peripheral nervous lesions and does not receive sufficient attention. In a series of 25 vitamin $B_{12}$-deficient persons with involvement of the nervous system, 14 had well marked cerebral symptoms and these are described in detail. All had pronounced slowing of mental processes and suffered from confusion. About half suffered a variable degree of depression and a few had delusions, five had hallucinations, three suffered from optic atrophy, three from epilepsy, two from dysphagia and two from mania.

These symptoms may appear well in advance of anemia or other neurologic signs. Except for two patients who had been demented for some years all of the patients recovered with intensive vitamin therapy.

The diagnosis is thoroughly discussed and the histopathology of the brains in two fatal cases is described; the lesions are similar to those of subacute combined degeneration of the cord with diffuse and focal areas of degeneration in the white matter and relatively little glial proliferation. F. E. HYTTEN

**Radioactive Vitamin $B_{12}$ in the Diagnosis of Neurological Disorders.** G. M. Berlyne, L. A. Liversedge and E. W. Emery. *Lancet* 1: 294, 1957.

Apart from its classical association with megaloblastic anemia, the diagnosis of subacute combined degeneration of the spinal cord is often in doubt. Possibly the most satisfactory method of diagnosis is to demonstrate that the patient cannot absorb vitamin $B_{12}$.

A technic is described, using vitamin $B_{12}$ labeled with $Co^{56}$ as a tracer, and illustrative cases are presented in which this method was used in diagnosis. There was a clear distinction between normal persons and those with known subacute combined degeneration. Doubtful cases could be confirmed or excluded with reasonable certainty. F. E. HYTTEN

**Vitamin $B_{12}$ in der Therapie der Poliomyelitis.** W. Daur. *Munch. Med. Wchnschr.* 98: 1366, 1956.

According to this report vitamin $B_{12}$ administration has a beneficial effect on the clinical picture of poliomyelitis, if given early enough. In doses of 1 mg (1,000 mcg) once or twice daily during the first week, less in subsequent weeks, there is said to be a diminution of the amyotrophy and a repression of neuralgia and myalgia. Unfortunately, the report presents no new data to confirm this recommendation.

S. O. WAIFE

*Many conflicting reports have appeared concerning the influence of vitamin $B_{12}$ upon growth rates and development of undernourished children. At the present time, it would seem that no conclusion can be reached concerning the role of the vitamin as a growth factor in humans.*

**The Influence of Vitamin $B_{12}$ and Aureomycin upon the Growth of Protein Deficient Children.** I. F. S. Mackay, S. J. Patrick, and D. Stafford. *J. Nutrition* 59: 155, 1956.

The purpose of this study was to examine the influence of vitamin $B_{12}$ and Aureomycin® upon the

growth of protein-deficient children, and at the same time to make an assessment of their nutritional status. There is disagreement as to the effect of dietary supplementation with vitamin $B_{12}$ on human growth, and conflicting results have been reported.

The children upon whom the observations were made in the present study lived in small rural communities in the southern part of the parishes of St. Thomas and St. Andrews in Jamaica, B.W.I. The people of these districts earn a rather poor subsistence by agriculture. Some fishing is done but this provides not so much food as currency. A group of children in an orphanage was also included in the study.

The study extended over a period of two years (1951–53). In the first half of the study, the growth of 2,012 children was measured. As a result of these measurements, 955 children were selected for supplementation on grounds of regular attendance at school. During the second half of the study supplements were administered to four groups which contained approximately equal numbers of children of each sex and growth type.

The results of this study present evidence for a slight positive effect of Aureomycin upon the rate of weight increase, but no effect upon the rate of height increase. The results show that vitamin $B_{12}$ has no positive effect upon weight or height increments. The nutritional status was observed to be low. Among the observations made were clinical examinations, growth measurements, biochemical measurements, and a direct dietary survey. There is no evidence to suggest that either Aureomycin or vitamin $B_{12}$ would be of practical value in alleviating the malnutrition found in such areas as the Caribbean.

The conflicting results on the effects of vitamin $B_{12}$ and/or Aureomycin on the rate of weight and height increases in growing children in various countries may be due to the different diets used by such countries, some of which may, and others of which may not, need vitamin $B_{12}$ supplementation. B. SURE

**The Relationship between Intrinsic Factor and the Intestinal Absorption of Vitamin $B_{12}$.** S. J. Baker and D. L. Mollin. *Brit. J. Haematol.* 1: 46, 1955.

The intestinal absorption of $Co^{58}$-labeled vitamin $B_{12}$ was studied in a number of patients with pernicious anemia in remission, by measuring the amount excreted in the faeces. Absorption of the vitamin increased linearly as the amount of intrinsic factor (from dried hog's stomach) was increased, until a point was reached where no further vitamin $B_{12}$ was absorbed regardless of the dose of intrinsic factor. The slope of the line relating vitamin $B_{12}$ absorption to the dose of intrinsic factor and the level beyond which no further absorption occurred showed wide individual differences. If a large intramuscular dose of vitamin $B_{12}$ was given shortly before or after the oral dose, absorption from the intestine was reduced. F. E. HYTTEN

**Intrinsic Factor Studies. IV. Selective Absorption and Binding of Cyanocobalamin By Gastric Juice in the Presence of Excess Pseudovitamin $B_{12}$ or 5,6-Dimethylbenzimidazole.** M. B. Bunge, L. L. Schloesser, and R. F. Schilling. *J. Lab. & Clin. Med.* 48: 735, 1956.

The authors describe studies with pseudovitamin $B_{12}$ which is a compound prepared by substituting adenine for 5,6-dimethylbenzimidazole in the vitamin $B_{12}$ molecule. They also studied the 5,6-dimethylbenzimidazole (DMBI) moiety by itself.

The following conclusions are made by the authors: (1) Neither pseudovitamin $B_{12}$ or DMBI compete with $B_{12}$ for the physiologic mechanisms affecting absorption of vitamin $B_{12}$ from the stomach. (2) Pseudovitamin $B_{12}$ is less effective than vitamin $B_{12}$ as a flushing agent in causing urinary excretion of absorbed radioactive vitamin $B_{12}$. (3) The *in vitro* binding of vitamin $B_{12}$ by gastric juice is a selective process showing a distinct preference for vitamin $B_{12}$ over pseudovitamin $B_{12}$ or DMBI. (4) The process of vitamin $B_{12}$ binding by serum, unlike that in gastric juice, does not manifest a selectivity for vitamin $B_{12}$ in the presence of excess pseudovitamin $B_{12}$. (5) The electrophoretic mobilities of vitamin $B_{12}$ binding substances in normal human gastric juice and an intrinsic factor concentrate are similar.

The authors also suggest that their data are in accord with the hypothesis that binding of vitamin $B_{12}$ is necessary for intrinsic factor activity, but binding alone is no criterion for the presence of such activity.

K. R. CRISPELL

## FOLIC ACID METABOLISM

*Folic acid serves as an essential co-factor in a variety of biochemical reactions involved in the biosynthesis of compounds necessary for the reproduction of cells. These reactions include the production and transfer of labile methyl groups; the interconversions and catabolism of certain amino acids; the production of vital purine compounds including thymidine, a part of the desoxyribonucleic acid molecule. Because of the primary role of the latter compound in cellular protein biosynthesis, it is not biosynthesis, it is not surprising that abnormalities are produced in the offspring of animals receiving a folic acid deficient diet.*

**Effect of 36-hour Period of Pteroylglutamic Acid Deficiency on Fetal Development in the Rat.** M. N. Nelson, H. V. Wright, C. D. C. Baird, and H. M. Evans. *Proc. Soc. Exper. Biol. & Med.* 92: 554, 1956.

Female rats of the Long-Evans strain were bred with normal males and given a diet deficient in pteroylglutamic acid (PGA) for 36 hours starting from the seventh to tenth day of pregnancy. For the remainder of the gestation, the diet was supplemented with adequate amounts of PGA. The offspring showed hydrocephalus, eye and cardiovascular abnormalities, cleft palate, harelip, syndactylism and clubfoot. Sensitivity to the deficiency and fetal death were highest if the

vitamin deficiency was instituted at the eighth day of pregnancy. It decreased rapidly thereafter. The variations in the sensitivity are comparable to those observed after irradiation of the rat embryo.

M. SILBERBERG

*Folic acid is converted to folinic acid (citrovorum factor by a reduction process and the addition of a formyl group.) It is the folinic acid coenzyme which functions as a biologic catalyst at the cellular level. The reaction of folic acid to folinic acid is enhanced by the presence of ascorbic acid or other reducing substances. Folinic acid is converted to folic acid in the upper intestine; however, the feeding of large amounts of ascorbic acid may reverse this process in the intestinal tract.*

**Metabolism of Folic Acid and Citrovorum Factor by Human Subjects.** R. E. Bleiler, D. Johnson, and H. T. Parsons. *J. Nutrition* 56:163, 1955.

From the evidence of various *in vitro* and *in vivo* experiments, folic acid is generally regarded as a provitamin, and citrovorum factor its active form, possibly the predominant form in all living tissue. Since the urinary excretion of citrovorum factor has been shown to be influenced by the ingestion both of pteroylglutamic acid and of ascorbic acid, the levels of intake of these two factors were varied for human subjects in this study, in the expectation that patterns of excretion might suggest significant relationships between folic acid and citrovorum factor in metabolism.

Citrovorum factor, on the average, accounted for one-fourth of the total folic acid activity in the urines of four human subjects on a weighed diet containing approximately 25 mg of ascorbic acid, and of 15 subjects on restricted self-administered diets containing approximately 100 mg of ascorbic acid. When folic acid had reached its maximum excretion four days after the initiation of a 1 mg daily dose of pteroylglutamic acid, citrovorum factor accounted for only one thirty-second of the folic acid activity of the urine. Citrovorum factor showed a pronounced but transitory increase in the urine in response to the dose of pteroylglutamic acid; a sharp decline started before folic acid had reached its maximum level of excretion. An excessive daily dose of ascorbic acid (750 mg) superimposed on the dose of pteroylglutamic acid restored the excretion of citrovorum factor to its previous high level. Various hypotheses to explain these relationships are discussed.

B. SURE

**Precursors of the Folic Acid-Active Factors of Blood.** G. Toennies, E. Usdin, and P. M. Phillips. *J. Biol. Chem.* 221:855, 1956.

Previous work by these authors had shown that under proper conditions human blood shows much higher folic acid activity than had been previously reported. Microbiologic assays indicated that a factor which was neither folacin, pteroylglutamic acid, pteroyltriglutamic acid nor folinic acid seemed to be the major component of the active material. Apparently a precursor material in the cells, termed FSP, could react with a plasma factor, PF, to form the active material, FS. It is suggested that FSP may be viewed as a storage form of folic acid-active compounds which is sequestered in the red cell and which becomes accessible to the action of PF as the red cell undergoes destruction at the end of its life.

M. K. HORWITT

*The relationship of folinic acid activity to that of other factors such as vitamin $B_{12}$ has been demonstrated in the areas of nucleoprotein and amino acid metabolism. Indeed there is evidence that vitamin $B_{12}$ deficiency may result in a lowering of the formation of folinic acid coenzyme. The low levels of vitamin $B_{12}$ in the diets employed in the following abstract may account for the lack of responses to folic acid administration.*

**Role of Citrovorum Factor in Nutritional Macrocytic Anaemia.** C. R. Das Gupta, J. B. Chatterjea, and R. N. Ray. *J. Indian M.A.* 26:85, 1956.

Seven cases of nutritional macrocytic anemia were treated with leucovorin, the synthetic citrovorum factor. The dose was 3 mg daily intramuscularly for six days, the subjects remaining on a low animal-protein diet. In five subjects the initial hemopoietic response was very good; it was fair in one, but no response was noted in the seventh. A well-sustained response was found in only two patients; in the others the improvement ceased at subnormal levels or else the anemia tended to recur. A second or third course produced a lesser response than was found at first. The responses to whole liver extract and high-protein diets suggest that these adjuvants "possibly contain accessory factors which greatly potentiate the hemopoietic activities of purified factors like vitamin $B_{12}$, folic acid and leucovorin."

S. O. WAIFE

*Acceleration of combined system disease in patients with pernicious anemia following the use of folic acid has been reported from several sources. Caution must be exerted in the recommendation of folic acid—containing vitamin preparations if the vitamin $B_{12}$ supply is inadequate.*

**The Role of Pteroylglutamic Acid and Related Compounds in Macrocytic Anemia.** G. H. Spray. *Proc. Brit. Nutrition Soc.* 15:119, 1956.

Nutritional macrocytic anemia appears to be due to dietary deficiency of folic acid. In India, where the disease responds to folic acid, the patients' diets were deficient in calories, animal protein, fresh fruit and green vegetables. In the southern United States, patients also had a low intake of animal protein and folic acid was effective against the anemia. In parts of Africa where megaloblastic anemia occurs, the diet is again low in animal protein, and rich in bulky carbohydrates. Thus, in all these areas there is a low intake of animal protein and probably also of other dietary sources of folic acid. A deficiency of vitamin $B_{12}$ may also occur

in this condition. PGA (pteroylglutamic acid) appears to be a specific in the therapy of sprue.

PGA corrects the megaloblastic anemia in infancy. A deficient intake of vitamin C probably plays a major part in the pathogenesis and vitamin $B_{12}$ is not effective in all patients.

The most plausible hypothesis advanced is that folic acid and vitamin $B_{12}$ take part in reactions leading to the synthesis of the nucleoproteins of blood cells from simple precursors. If vitamin $B_{12}$ is deficient, as in pernicious anemia, there is an excessive demand for folic acid, which keeps the reactions going by "mass action." This ultimately causes a deficiency of folic acid. When the lack of vitamin $B_{12}$ is compensated, the demand for folic acid falls and the deficiency disappears. Large doses of folic acid increase blood formation by raising the concentration of pyrimidines or purines, so that the later reactions can proceed despite the shortage of vitamin $B_{12}$. This process uses up the remaining small stores of vitamin $B_{12}$, causing exacerbation of the nervous degeneration, because vitamin $B_{12}$ is apparently essential for the integrity of the nervous system as well as of the hematopoietic system. Sooner or later insufficient vitamin $B_{12}$ is left for proper blood formation, even in the presence of large amounts of PGA. This may explain why patients with pernicious anemia on PGA may relapse hematologically.

B. SURE

*Although gout is regarded as a metabolic disease due to abnormalities in purine metabolism and associated with an "expanded" uric acid pool, there is little knowledge as to the actual mechanisms involved in production of the urate disturbance. Uric acid may accumulate as a result of nucleoprotein degradation or it may be synthesized from simple carbon compounds or both. The favorable response observed occasionally with low purine diet therapy would tend to favor the former.*

**Metabolism of Citrovorum Factor and Folic Acid in Gout; Urinary Excretion Studies.** W. W. Faloon and S. Dilg. *J. Lab. & Clin. Med.* 48:399, 1956.

The authors point out that Spilman has reported that $C^{14}$ administered as formate appears in higher concentrations and more rapidly in the urinary uric acid of gouty persons than in normals. Spilman theorized that hyperuricemia in these individuals may be a result of increased urate formation by non-nucleoprotein pathways. The role of Citrovorum Factor (C.F.) or a closely related compound in purine and uric acid formation has been suggested in the demonstration that such compounds may act as transformylating agents in the formation of purines. The studies reported are on the urinary excretion of Folic Acid (FA) and Citrovorum Factor (C.F.) following the administration of these substances to patients with gout and normal healthy controls and so-called "hospital normals." In addition, the effect of ascorbic acid which has been shown to stimulate conversion of pteroylglutamic acid to C.F. was investigated.

The average urinary C.F. in gouty patients was significantly less than in normal subjects following either PGA orally or C.F. intramuscularly.

It should be pointed out that there was a wide range of urinary C.F. excretion in healthy normals and there was an overlap with the gouty patients. It was the average urinary C.F. excretion which was decreased. FA excretion following oral PGA was, however, not different in the two groups. C.F. excretion did not correlate with blood ascorbic acid concentrations, although patients with gout exhibited lower average concentrations. Gouty individuals responded normally to ascorbic acid administration with increased urinary C.F. The authors feel that their data under the conditions of the study, suggest either increased C.F. utilization or renal retention of C.F. is present in gouty patients.

K. R. CRISPELL

## ITEMS OF GENERAL INTEREST

**Nutrition and Behavior—Psychologic Changes in Acute Starvation with Hard Physical Work.** J. Brožek. *J. Am. Dietet. A.* 31:703, 1955.

This is a report of observations on the alteration in man's performance capacity under stress of starvation. In men studied during four days of starvation the author reports the following findings: "There was a dramatic decrease in endurance, as measured by running on the treadmill to exhaustion. Statistically highly significant deterioration was noted in the tests of speed and in eye-hand coordination. Intellective tests showed little or no impairment. In the Minnesota Multiphasic Personality Inventory there was a large increment on the psychoneurotic scales. Tiredness, muscular weakness (with no counterpart in measured strength), and soreness of muscles dominated the subjective symptoms."

These conclusions were based on data collected through cooperation of 12 "normal young men" who went without food for four and a half days on two occasions at six-month intervals. Results presented in the paper (except for intellective tests) refer to the second experiment. In addition to the stress of starvation, an activity program was imposed through walking for several hours daily on an inclined motor-driven treadmill (3.5 m.p.h., 10 per cent grade) equivalent to an average expenditure of 550 cal per hour. According to the author, it was estimated that the average calorie deficit imposed by the starvation-exercise regime was 14,500 by the evening of the fourth day.

Physiologic responses of these 12 men are represented in tabular form as group means without indications of individual deviations. On the basis of these means, the author calls attention to the weight loss of 4.6 kg (about 10 lb) on the morning of the fourth day. Nearly 40 per cent of this loss had occurred within the first 24 hours, probably indicating excessive loss of body water. "Blood sugar at work" changed from 80 mg/100 ml initially to a mean of 62 on the fourth day. Comparable values for the intervening days were "normal range,"

53, and 59 on the first, second, and third days of starvation. The pulse rate, counted during the last five minutes of the first hour of walking, changed from a mean of 129 beats/min, before starvation, to 152 after four starvation days. There was marked reduction in the time of running to exhaustion on a treadmill (240 minutes initially and 134 finally).

The psychomotor battery included: (1) two tests of strength, i.e., handgrip and back pull dynamometer; (2) three tests of speed—"two-plate" tapping, speed of coordinated hand-and-arm movements, and a test of choice reaction time; (3) a test of eye-hand coordination; and (4) a measure of body sway. Mean values for these psychomotor tests are given for all subjects on a control day and on the fourth day of starvation. The author calls attention to decrements in all three tests of speed, adjudged highly significant statistically (data on statistical treatment do not appear in this paper). No decrement in hand grip was observed and the decrement in back pull (7.9 kg) did not reach a level of statistical significance. "Statistically highly significant deterioration" was obtained in coordination, as measured by number of errors and duration of errors in pattern tracing. The measure of body sway revealed a significant decrement when values averaging 11.5 on the control day were compared with those averaging 16.3 on the fourth day.

Conclusions concerning significance of findings (but no actual data) are given for tests of sensory function, flicker fusion frequency; tests of intellective function; and tests of personality as measured by the Minnesota Multiphasic Personality Inventory.

The author indicates that the study herein reported will provide a useful background for further work currently under way in the same laboratory on the reduction of deterioration of fitness which can be obtained by feeding emergency rations. An extensive bibliography documents the methods and some of the findings presented in this paper.

In the opinion of the reviewer this article would have been improved by inclusion of some description of the extent of variation of the reported means, together with a summarization of actual statistical values. The paper was, however, originally given at an annual meeting of the American Dietetic Association and undoubtedly conformed with the type of presentation requested by that organization. J. M. SMITH

**Experimental Hypertension in Rats Produced by Dietary Condiments.** L. W. Roth and J. Blair. *Proc. Soc. Exp. Biol. & Med.* 94: 619, 1957.

For a period of one year female rats, weighing 200 to 250 g, were fed a diet supplemented with (a) 2 per cent pepper, (b) 1 per cent each of mustard and ginger, and (c) 2 per cent pepper, 1 per cent mustard and 1 per cent ginger. The supplements caused statistically significant hypertension, the greatest rise occurring after feeding the diet enriched with all three condiments. Microscopically, the kidney failed to show changes characteristic of any drug action, and the findings were essentially negative. The hypertension persisted for months after discontinuation of the supplement.

M. SILBERBERG

**The Volume of Distribution of High Molecular Weight Dextran and Its Relation to Plasma Volume in Man.** A. B. Craig, and C. Waterhouse. *J. Lab. & Clin. Med.* 49: 165, 1957.

The authors present the results of studies on the use of a high molecular weight dextran for the measurement of plasma volume in man. The desirability of using this substance is that it does not form a complex with plasma proteins. It is hoped that even in pathologic states, the macromolecules of dextran would be retained in the intravascular system.

Preliminary work with low molecular-weight dextran demonstrated the unsuitability of this material for plasma volume measurement. Studies were carried out in eleven healthy volunteers and "the characteristics and volume of distribution make it likely that dextran is diluted in the plasma volume." K. R. CRISPELL

**The Relative Volumes of Distribution of $I^{131}$ Tagged Albumin and High Molecular Weight Dextran in Normal Subjects and Patients with Heart Disease.** J. R. Jaenike, B. F. Schreiner, Jr., and C. Waterhouse. *J. Lab. & Clin. Med.* 49: 172, 1957.

This study is concerned with the relative volumes of distribution of $I^{131}$—tagged albumin and high molecular—weight dextran in ten normal subjects and in fifteen patients with cardiac failure and edema.

In all groups studied the volume of distribution of tagged albumin was significantly greater than that of the dextran. The discrepancy was greater in patients with heart disease than in normal subjects. The data presented suggest that albumin "escapes" from the circulation in larger amounts than dextran, and that this loss occurs mainly during the period of intravascular mixing. This phenomenon appears to be exaggerated in heart disease. "This results in a falsely high calculated plasma volume based on the dilution of albumin." This is true from the authors' data but we still do not know if either method actually measures plasma volume. It may be best to refer to the "dextran" space of the $I^{131}$ albumin" space rather than the plasma volume. Also, it has been shown that radiation of the albumin by the labeled iodine may "alter" the albumin so that it has a faster disappearance rate from plasma. This is an excellent study and gives one more reliable method of measuring changes in an "intravascular space."

It is of interest that both methods showed an expansion of plasma volume in congestive heart failure, with edema. K. R. CRISPELL